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In Vitro Anti Sickling Activity of *HyphaeneThebaica* Fruits (Doum) Extract on Sickle Cells

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Abstract

Background: *Traditional medicine had been used alongside synthetic pharmaceutical products to enhance health management. Due to the high mortality rate of sickle cell patients, previous studies have been indicated that some medicinal plants have shown an anti-sickling activity, which indicates a new therapeutic way to manage people who are affected by these disorders. The current study aimed to assess in vitro-anti-sickling activity of Hyphaene thebaica (H thebaica) (Doum) fruit.*

Materials and methods: *Blood samples used in the evaluation of the anti-sickling activity of Hyphaene Thebaica Fruits extract in this study was taken from patients known to had Sickle cell disease (HB-SS) attending the Sickle Cell Clinic in Khartoum state. Emmel test was used to assess anti-sickling activity of this plant.*

Result: *A significant increase in the percentage of unsickled Red blood cells with p-value* ***< 0.05****was observed after incubation of*

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RBCs with 2% sodium metabisulfite in the presence of three different concentration (1000, 500 and 250) µg/ml of Hyphened thebaica for two type of extraction (aqueous and methanol) extract.

Conclusion: *This study approved that H.thebaica (Doum) fruit extract had a strong anti- sickling activity ; it could be used for management of sickle cell disease.*

Keywords: In vitro, Anti-sickling activity, Haphaene Thebaica, Percentage of unsickled red blood cells, Sickle cell disease.

Background

Sickle cell disease (SCD) is a genetic disorder caused by single point mutation in the sixth codon of the beta globin chain which affect the amino acid constituent of goblin chain leading to change in the hemoglobin affinity toward oxygen and also changes the hemoglobin solubility under low oxygen pressure. This mutation and their consequences of symptoms are the reasons of the high mortality rate in SCD , especially in children of developing country such as Sudan where the chemotherapy is choice of the good income parents and alongside the adverse effect of this medications so there are emerging needs for traditional drugs which are consider safe treatments with reasonable price.[1,2]

Some of medicinal plants are thought to be responsible for observed antisickling action for their high contain of phenolic compounds and antioxidant nutrients. Antioxidants (scavengers of free radicals) are thought to be major components of these antisickling action thus different antisickling agents have different degrees of effect according to amount of antioxidant. [3] So that the higher antioxidant property of an antisickling agent enables it to reduce oxidative stress that contributes to sickle cell crisis. *Hyphaene thebaica* common name is African doum palm it is a type of desert palm tree with edible oval fruit which belongs to the family Arecaceae. Doum palm is native to the northern half of Africa; it tends to grow along the Nile River in Egypt and Sudan in the areas which contain ground water. The extracts of *Hyphaene thebaica* was used in treatment of hypertension, bilharzias and as a haematinic agent. doum fruit extracts contain high amount of

flavonoids, phenols used as antioxidant and antibacterial activities which can alleviate the adverse effects of oxidative stress.[4]

Phytochemical components show medicinal values of these plants, which produce definite and various physiological action on human body. Some of the most important of these components are flavonoids, alkaloids and phenolic compounds [5].

Phytochemicals are extensively present at different level in many medicinal plants and used in herbal medicines to treat different ailment such as cough, diabetes, hypertension, cancer, and various types of bacterial infections. It is also used to treat sickle cell crises associated morbidities among the less privileged classes of the society [6].

Doum Palm (Hyphaene thebaica)

Hyphaene thebaica its common name is African doum palm it is a type of desert palm tree with edible oval fruit which belongs to the family Arecaceae. The doum palm is native to the northern half of Africa. It grows in the west from Mauritania and Senegal, and east to Egypt, Kenya and Tanzania.

It tends to grow along the Nile River in Egypt and Sudan in the areas which contain ground water. The various extracts of *Hyphaene thebaica* used in the treatment of hypertension, bilharzias and as a haematinic agent. Several studies have recorded that doum fruit extracts contain high amount of flavonoids, phenols used as antioxidant and antibacterial activities which can alleviate the adverse effects of oxidative stress [7].

Chemical composition of Doum fruit

Doum fruit has a high-quality protein varied between 2.86 and 5.01%, high proportion of lysine and cysteine of crude protein varied between 4.09–4.16% and 0.2–1.62%, respectively, the limited amino acid threonine, crude fat varied between 1.2 and 8.4%, crude fiber varied between 52.26 and 66.5%, the most important carbohydrates component was mannose varied between 13 and 75.9%, also the presence of calcium, magnesium, potassium, iron sodium and negligible amount of nickel, cobalt and molybdenum. Phytochemical compounds of doum fruit such as tannins, saponin, steroids, glycosides, flavonoid,

terpenes and terpinoids were found at low and moderate concentrations [7].

Chemical structure of doum fruit phenolic compounds

Different total soluble phenols values in doum were published in different studies; it ranged from 45.08 to 64.90 mg GAE/g DW . While it recorded the highest values in pitted doum fruit extracts varied from 1 16.26 to 139.48 mg GAE/g DW. The bioactive potential of fruits and vegetables attributed to their high content of polyphenols [28 The most abundant phenolic compounds recorded in doum were metoxicinnamic acid, sinapic acids (hydroxycinnamic acids), chlorogenic acid, catechin, p-hydroxybenzoic acid, vanillic acids, 3,4 di hydroxycinnamic acid, caffeic acid, 2-hydroxycinnamic acid, Epicatechinand cinnamic acid, respectively Doum pulps exhibited higher caffeic acid contents in comparison to the domestic fruits. The highest four concentrations of phenolic compounds in doum fruit aqueous extracts were found to be 3-OH tyrosol, E-vanillic acid, catechin and chlorogenic acid, while the lowest were of alpha-coumaric acid, cinnamic acid, p-coumaric acid and coumarin [7].

Total flavonoids content and compounds of Doum fruits

The total flavonoids content in different extracts of doum fruit extracts varied widely ranging from 24.04 to 47.17 mg rutin/g DW . Similar results found that the content of flavonoids (mg/g) of fruits of H. thebaica, in the quercetin equivalent was 46.28 mg/g DW . HPLC analysis of aqueous doum fruit extracts showed 11 flavonoid compounds . The highest concentrations were quercetin, hesperetin, naringin and rutin compounds . Five flavone glycosides were isolated 4 and identified from doum fruits namely, luteolin 7-O-ßglucuronoide, apigenin 7- O-ß-glucuronoide, luteolin O-ß-glycoside, luteolin 7-O-rutinoside.[7]

Materials and methods

This was experimental done in Khartoum state from September 2019 to January 2020. The study populations were ten sickle cell anemia samples obtained from diagnosed sickle cell disease patients (HBSS).

Inclusion and exclusion criteria

Samples had been collected from sickle anemia patient, including both genders in different age. Patients already diagnosed with other disorder and hereditary hemoglobinopathy was excluded from the study.

Preparation of the aqueous extract of doum fruits

40 g of the doum fruits was extracted by soaking in 200 ml hot distilled water for about four hours with continuous steering. Then 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 [5].as followed:

(Weight of dish with extract – empty weight) X 100 / 2

Preparation of methanol extract

100 g of the plant sample was coarsely powdered using mortar and pestle. Then soaked in absolute methanol. Extraction carried out for three 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 9.92 % were calculated[5] as follows:

Weight of extract obtained / weight of plant sample X 100.

Methodology

Washing of RBCs

About 4 ml Ethylene diamine tetra acetate (EDTA) blood samples had been obtained from patients then centrifuged at 3000 rpm for 10 minutes to remove the plasma. The resulting packed erythrocytes had been washed 3 times with 1 ml sterile normal saline per 5 ml of blood. The samples had been centrifuged each time to remove the supernatant.

Procedure for anti-sickling activity evaluation

Three diluted solutions in normal saline had been prepared from the stock solution of plant extract as follows (250, 500, and 1000 µg/ml). Emmel test: Washed erythrocyte had been mixed with an equivalent volume of 2% sodium metabisulfite (Na2O5S2). 10 µl from the above

mixture had been spotted on a microscope slide then 10 µl from the plant extracts had been added and mixed with the blood mixture. 10µl normal saline had been added to one of the slides instead of the plant extract which served as control; all the slides had been covered with a cover slip. Paraffin had been applied to seal the edges of the cover completely to exclude air (hypoxia), and then, slides had been incubated for one hour minutes. Each slide had been examined under oil immersion light microscope, and red blood cells (RBCs) had been counted in five different fields of view across the slide. The numbers of both sickled and unsickled blood cells had been determined, and the percentage of unsickled cells had been calculated using the formula:

{(%) unsickling = Number of unsickling cells × 100/total red blood cells}.

Statistical analysis

Data had been reported as mean ± standard deviation (S.D), independent T test had been used to compare between mean value of the control and different concentration of extracts and also to calculated p-value. P-values less than 0.05 were considered significant.

Result

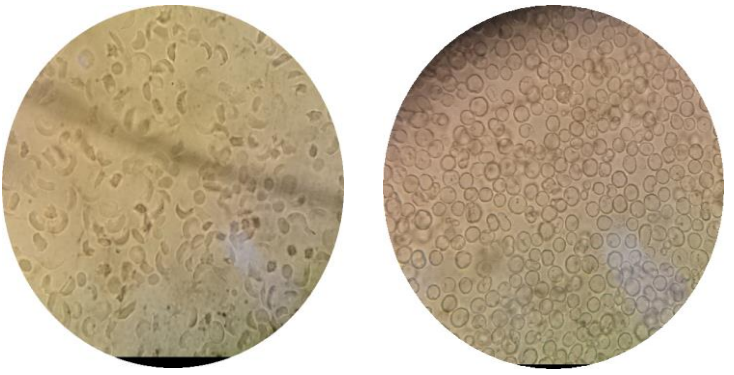
The mean % of unsickel cells when Incubated with *H. Thebica* aqueous extract in different concentrations were *(*96.6 ± 3.7 , 95.3 ± 5.2 and 96.3 ± 4.9)Respectively when compared with mean % of control (59.7± 31.3), The results were statistically significant with p-value (0.005, 0.006 and 0.005) respectively. There were no statistical significant different between aqueous extractions of different concentration (1000, 500 and 250 µg/ml) p-value were (0.52, 0.89and 0.61) respectively.

Mean % of unsickle cells when incubated with *H. Thebica* methanol extract in different concentrations (1000,500 and 250µg/ml) were *(*97.9 ± 2.8, 98.1 ±2 and96.4 ± 3.8 *)* respectively, when compared with mean % of control (59.7± 31.3), results were statistically significant with p-value (0.004, 0.004 and 0.005) respectively.

There was no statistically significant difference when compared the three different concentration of methanol extraction of Doum (1000, 500 and 250 µg/ml) p-value were (0.94, 0.27 and 0.24) respectively . Also there was no statistical significant difference observed when compared the mean % of un-sickle cells of the three aqueous extraction

concentration versus the three methanolic extraction concentration, for all p-value >0.05

Figure[1]: sickle versus un sickle cells in control and 1000 µg/ml aqueous extraction of Doum



Control **test in1000 µg/ml aqueous extraction**

Table1: Multiple Comparisons of mean of % of un sickle cells in aqueous extraction and control

(I)	(II)	Mean (I)	Mean (II)	P. value
Control	A 1000µg/ml		96.6 ± 3.7	0.005
	A 500µg/ml	59.7 ± 31.3	95.3 ± 5.2	0.006
	A 250µg/ml		96.3 ± 4.9	0.005

A ; Aqueous extraction , p-value <0.05 : significant difference

Table 2: Multiple Comparisons of mean of % of un sickle cells in aqueous extraction

(I)	(II)	Mean (I)	Mean (II)	P. value
A 1000µg/ml	A 500µg/ml	96.6 ± 3.7	95.3 ± 5.2	0.520
	A 250µg/ml		96.3 ± 4.9	0.892
A 500µg/ml	A 250µg/ml	95.3 ± 5.2	96.3 ± 4.9	0.611

A ; Aqueous extraction , p-value <0.05 : significant difference

Table 3: Multiple Comparisons of mean of % of un sickle cells in methanolicextraction and control

(I)	(II)	Mean (I)	Mean (II)	P. value
Control	M 1000µg/ml		97.9 ± 2.8	0.004
	M 500µg/ml	59.7 ± 31.3	98.1 ± 2.4	0.004
	M 250µg/ml		96.4 ± 3.8	0.005

M; Methanolic extraction, p-value <0.05: significant difference

Table 4: Multiple Comparisons of mean of % of un sickle cells in methanolicextraction of Doum

(I)	(II)	Mean (I)	Mean (II)	P. value
M 1000µg/ml	M 500µg/ml	97.9 ± 2.8	98.1 ± 2.4	0.937
	M 250µg/ml		96.4 ± 3.8	0.272
M 500µg/ml	M 250µg/ml	98.1 ± 2.4	96.4 ± 3.8	0.241

M; Methanolic extraction, p-value <0.05: significant difference

Table 5: Multiple Comparisons of mean of % of un sickle cells in aqueous and methanolic extraction

(I)	(II)	Mean (I)	Mean (II)	P. value
A 1000µg/ml	M 1000µg/ml	96.6 ± 3.7	97.9 ± 2.8	0.809
	M 500µg/ml		98.1 ± 2.4	0.794
	M 250µg/ml		96.4 ± 3.8	0.974
A 500	M 1000µg/ml	95.3 ± 5.2	97.9 ± 2.8	0.628
	M 500µg/ml		98.1 ± 2.4	0.615
	M 250µg/ml		96.4 ± 3.8	0.833
A 250µg/ml	M 1000µg/ml	96.3 ± 4.9	97.9 ± 2.8	0.770
	M 500µg/ml		98.1 ± 2.4	0.755
	M 250µg/ml		96.4 ± 3.8	0.985

A; Aqueous extraction, M; Methanol extraction, p-value <0.05 : significant difference

Discussion

This study demonstrates that Anti-sickling activity of aqueous and methanol extract of Doum fruit; the effect of plant crude extracts in different concentration (100, 500 and 250 µg/ml) on sickle cell after the incubation for one hour, the results were highly significant all p-values 0.05 >this result in concordance with study done by HS El-

Beltagi, Mohamed HI, etal (2018) [4]; demonstrate that doum Fruit extraction had bioactive potential attributed to their high content of antioxidant (phenols compound an flavonoids) ; antioxidant activity had direct proportion to anti sickling activity[8,9].

Multiple Comparisons of mean of % of un sickle cells in aqueous extraction in different concentrations confirmed that no statistically different between Doum extraction of different concentrations. The results were disagreed with study done by Mohamed AA, et al (2010) [9]; they found that the aqueous doum extract exhibited antioxidant activity increased with the increased of extract concentration in this study all concentration had similar anti sickling activity with no statistical significant differences this may be due to different in experiment variables .

Time course for antisickling activity of the aqueous and methanol extract of Doum fruit (60 minute incubation) carried out on blood samples of sickle cell disease revealed significant effect on % of un sickle cell when compared with control. The majority of antioxidants, both natural and synthetic, are phenolic compounds [10, 11]. In this study Doum extract had strong antisickling activity, could be due to the high content of antioxidant.

According to our information, no previous study was done to determine the activity of Doum extract on sickle cells as this is the first study.

Conclusion

This study approved that H.thebaica (Doum) fruit extract had a strong anti sickling activity so it could be used for management of sickle cell disease and also it is consider cheap and one of the abundant fruits in Africa. Further in vivo studies are recommended to evaluate the effect and to determine the mode of action of this plant.

Abbreviations

HB-SS: Homozygous sickle cell hemoglobin; SCD: Sickle cell disease; EDTA: Ethylene diamine Tetra-acetate;Na2O5S2: Sodium metabisulfite; RBCs: Red blood cells; SD: Standard deviation; H thebaica: Hyphaene thebaica.

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