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Effect of Crude Mesocarp Extract of *Hyphaene thebaica* (doumpalm) on White Blood Cells and Differential Leucocytic Count in Wistar Albino Rats

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The effect of crude mesocarp extract of *Hyphaene thebaica* on white blood cell and differential leucocytic counts in wistar albino rats was evaluated. Fresh mesocarp of *Hyphaene thebaica* collected were ground into fine powder, extracted by reflux method and subjected to phytochemical screening for biochemical principles. The quantitative phytochemical screening revealed the presence of low level of tannins, steroids and moderate level of saponins, carbohydrates, cardiac glycosides, flavonoids, Terpenes and Terpinoids. The elemental analysis of the extract revealed the presence of calcium, magnesium, potassium, iron and sodium in moderate concentration. The amount of zinc and silicon were low whereas, nickel, cobalt, molybdenum, arsenic and lead were negligible. The crude mesocarp extract administered at the dosages of 400, 600 and 800 mg kg⁻¹ significantly (p<0.05) increased cells count of Lymphocytes, Monocytes, Eosinophils and Basophils during four weeks of administration. The extract did not have any significant effect on Neutrophils count throughout the period of treatment. This finding validates the folkloric application of the mesocarp extract of *Hyphaene thebaica* in the management of parasitic and viral infections in North eastern region of Nigeria.

Key words: Crude mesocarp, *Hyphaene thebaica*, differential leucocytic count, wistar albino rats

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INTRODUCTION

Herbal preparations has been applied to boost wide range of primary health care delivery due to biochemical contents of plants in Africa and other parts of the world (Elujoba *et al.*, 2005). *Hyphaene thebaica* belongs to the family Arecaceae commonly known as doum palm, Dum Nut or gingerbread tree. Important economic species also occur in India and other parts of Asia (Brunken *et al.*, 2008). Roots, fruits and seeds of doum palm are used for the management of jaundice, intestinal colic, hematuria, inguinal hernia bilharzias, hypertension and sore eyes respectively in livestock. The mesocarp is credited with diuretic properties (Owolarafe *et al.*, 2007) and also have been found to possess antioxidant property (Hsu *et al.*, 2006).

Phytochemicals are essential elements with protective effect that are required by humans and animals to sustain life. Recent researchers found that Phytochemicals can protect humans against diseases such as hypertension, cancer, diabetes and various forms of microbial infections (Di Carlo *et al.*, 1993).

The level of leucocytes in blood is an important pointer to physiological and pathological status of an individual (Schalm *et al.*, 1975). The concentration of these cells in blood can be increased or decreased by the ingestion of some medicinal plants (Ajagbonn *et al.*, 1999). The aim and objectives of this study is to evaluate the Phytochemical, elemental contents and effects of crude mesocarp extract of *Hyphaene thebaica* on white blood cells and differential leucocytic count in wistar albino rats.

MATERIALS AND METHODS

Plant collection and identification: Fresh mesocarp of *Hyphaene thebaica* was bought in September 2012 from Gamboru market, Borno state, North eastern, Nigeria. The seeds were authenticated by a taxonomist at the Department of Biological Science, University of Maiduguri. Voucher specimen of this plant was kept in the toxicology laboratory, University of Maiduguri for reference.

Preparation of crude *Hyphaene thebaica* mesocarp extract: Fresh mesocarp of *Hyphaene thebaica* collected were ground into fine powder and stored in a glass container. One hundred and fifty grams of aqueous product are prepared by reflux method from three hundred and fifty grams of initial powdered sample. The aqueous seed extract obtained was then concentrated, labeled and stored in a refrigerator at 4°C.

Phytochemical analysis of aqueous mesocarp extract:

Phytochemical screening for tannins, anthraquinones, flavonoids and carbohydrates was carried out using the method described by Trease and Evans (1989, 1997) while glycosides, alkaloids, reducing sugars, monosaccharides, ketones, pentoses and terpenes by Sofowora (1982), Odebiyi and Sofowora (1978) and saponins by Harborne (1973).

Test for Tannins (Ferric chloride test): Two milliliter of the crude solution of the extract was added to few drops of 10% Ferric chloride solution (light yellow). The occurrence of blackish blue color shows the presence of gallic tannins and a green-blackish color indicates presence of catechol tannins.

Test for saponins (frothing test): Three milliliters of the crude solution of the extract was mixed with 10 mL of distilled water in a test-tube. The test-tube was stoppered and shaken vigorously for about 5 min; it was allowed to stand for 30 min and observed for honeycomb froth which is indicative of the presence of saponins.

Test for alkaloids: One gram of the extract was dissolved in 5 mL of 10% ammonia solution and extracted with fifteen milliliter of chloroform. The chloroform portion was evaporated to dryness and the resultant residue dissolved in 15 mL of dilute sulphuric acid. One quarter of the solution was used for the general alkaloid test while the remaining solution was used for specific tests.

Mayer's reagent (or bertrand's reagent): Drops of Mayer's reagent was added to a portion of the acidic solution in a test tube and observed for an opalescence or yellowish precipitate indicative of the presence of alkaloids.

Dragendorff's reagent: Two milliliters of acidic solution in the second test-tube was neutralized with 10% ammonia solution. Dragendorff's reagent was added and turbidity or precipitate was observed which was indicative of presence of alkaloids.

Tests for carbohydrate (Molisch's test): Few drops of Molisch's solution was added to 2 mL of aqueous solution of the extract, thereafter a small volume of concentrated sulphuric acid was allowed to run down the side of the test tube to form a layer without shaking. The interface was observed for a purple colour which is indicative of positive for carbohydrates.

Tests for carbohydrate (Barfoed's test): One milliliter of aqueous solution of the extract and 1 mL of Barfoed's reagent were added into a test-tube, heated in a water bath for about 2 min. Red precipitate shows the presence of monosaccharides.

Standard test for combined reducing sugars: One milliliter of the crude solution of the extract was hydrolyzed by boiling with 5 mL of dilute hydrochloric acid. This was neutralized with sodium hydroxide solution. The Fehling's test was repeated as indicated above and the tube was observed for brick-red precipitate that indicates the presence of combine reducing sugars.

Standard test for free reducing sugar (Fehling's test): Two milliliter of the crude aqueous solution of the extract in a test tube was added 5 mL mixture of equal volumes of Fehling's solutions I and II and boiled in a water bath for about 2 min. The brick-red precipitate indicates the presence of reducing sugar.

Test for ketone: Two milliliter of crude aqueous solution of the extract was added a few crystals of resorcinol and an equal volume of concentrated hydrochloric acid and then heated over a spirit lamp flame and observed for a rose coloration, that shows presence of ketone.

Test for pentoses: Two milliliter of the aqueous solution of the extract was added an equal volume of concentrated hydrochloric acid containing little phloroglucinol. This is heated over a spirit lamp flame and observed for red coloration, indicative of presence of pentoses.

Test for Phlobatannins (Hydrochloric acid test): Two milliliter of the crude aqueous solution of the extract was added dilute hydrochloric acid and observed for red precipitate that indicates presence of Phlobatannins.

Test for cardiac glycosides: Two milliliter of the crude aqueous solution of the extract was added 3 drops of strong solution of lead acetate. This was mixed thoroughly and filtered. The filtrate was shaken with 5 mL of chloroform in a separating funnel. The chloroform layer was evaporated to dryness in a small evaporating dish. The residue was dissolved in a glacial acetic acid containing a trace of ferric chloride; this was transferred to the surface of 2 mL concentrated sulphuric acid in a test tube. The upper layer and interface of the two layers were observed for bluish-green and reddish-brown coloration, respectively which indicates the presence of cardiac glycosides.

Test for steroids (Liebermann-burchard's test): The amount of 0.5 g of the crude aqueous extract was dissolve in 10 mL anhydrous chloroform and filtered. The solution was divided into two equal portions for the following tests. The first portion of the solution above was mixed with one mL of acetic anhydride followed by the addition of 1 mL of concentrated sulphuric acid down the side of the test tube to form a layer underneath. The test tube was observed for green coloration indicative of steroids.

Test for steroids (Salkowski's test): The second portion of solution above was mixed with concentrated sulphuric acid carefully so that the acid formed a lower layer and the interface was observed for a reddish-brown colour indicative of steroid ring.

Test for flavonoids (Shibita's reaction test): One gram (1g) of the crude aqueous extract was dissolved in methanol (50%, 1-2 mL) by heating, then metal magnesium and 5-6 drops of concentrated hydrochloric acid were added. The solution when red is indicative of flavonols and orange for flavones.

Test for flavonoids (Pew's test): To five milliliter of the crude solution of the water extract was added 0.1 g of metallic zinc and 8 mL of concentrated sulphuric acid. The reaction mixture was observed for red color indicative of flavonols.

Test for anthraquinones (Borntrager's reaction for free anthraquinones): One gram of the powdered seed was placed in a dry test tube and 20 mL of chloroform was added. This was heated in steam bath for five minutes. The extract was filtered while hot and allowed to cool. To the filtrate was added equal volume of 10% ammonia solution. This was shaken and the upper aqueous layer was observed for bright pink coloration which is an indication of the presence of Anthraquinones. Control test were done by adding 10 mL of 10% ammonia solution in 5 mL chloroform in a test tube.

Elemental analysis: The elemental content of the extract was determined using the standard calibration curve method (Sunderman, 1973; Kolthoff and Elving, 1976). Zero point five grams of air dried sample in an evaporating dish was placed in oven at 80°C and dried to a constant weight. The sample is placed in a weighing crucible and ashed at 500°C in a hot spot furnace for three hours. The ashed material is prepared for determination of trace element. A portion of Zero point five grams of the ashed sample is digested by heating

for two minutes with a mixture of 10 mL each of Nitric acid (HNO₃), Hydrochloric acid (HCl) and a perchloric acid in a 500 mL flask. The aliquot obtained is mixed in a 10 mL 2 M HNO₃ and 30 mL of distilled water in a 100 mL volumetric flask. The volume is made up to the mark with distilled water. Blank sample and standard solution for the various elements were similarly done. All samples are placed in a plastic container and stored in a refrigerator maintained at 4°C prior to analysis. Flame Emission Spectrometer (FES) (GallenKamp FGA 330) is used to determine sodium and potassium concentration. The other elements, Magnesium, Calcium, Iron, Lead, Zinc, Manganese, Cadmium, Copper and Arsenic were determined by Atomic Absorption Spectrometry (AAS) with SPG Unicam model No. 1 at the appropriate wavelength, temperature and lamp current for each element.

Blood sample collection: Blood samples were collected from the tail vein of the rats by snipping part of the tail for the determination of red blood cells, packed cell volume, hemoglobin concentration and white blood cells counts.

Determination of red blood cells (RBC) count: The method described by researchers was used for the red blood cells count determination. This is an improved Neubauer method. The erythrocyte diluting pipette was used to draw blood from the tail vein to exactly 0.5 marks. The tip of the pipette was wiped free of blood before inserting into the erythrocyte diluting fluid and the fluid drawn into the pipette up to the 101 mark above the bulb. The pipette was gently rotated and allowed to stand for 2 min. The first few drops from the pipette were discarded before being used to charge the counting chamber. The ruled areas of the hemacytometer were thoroughly and carefully cleaned to remove grease. The cover slip was then placed on the counting chamber which was thereafter charged with the fluid from the pipette. The chamber was left for 2 minutes. And cells in 5 of 25 small squares were counted under 40x objective of light microscope. The number of the red cells counted were multiplied by ten thousand (10,000) to give number of the red blood cells in million per cubic millimeter (or $\times 10^6 \text{mm}^3$) (Coles, 1986).

Determination of the packed cell volume (PCV): Blood from the tail vein of the rat was allowed to run into microhematocrit tube by capillary action until the tube is about three-quarter full. The end of the tube in contact with the blood was sealed with Plasticine and placed in a

micro-haematocrit centrifuged operated at the rate of 3,000 Revolutions per Minute (rpm) for 5 min, thereafter, the capillary tube was placed in a micro-haematocrit reader and the PCV read and expressed as percentage (Coles, 1986).

Determination of the Hemoglobin concentration (Hb): Colorimetric method for the determination of hemoglobin concentration was used (Dacie and Lewis, 1994; Coles, 1986). To 5 mL of Drasbsken's solution in a series of test tubes was added 0.2 cm³ of blood and allowed to stand for 3 min to allow the blood to react with the cyanide solution properly. The colorimeter was warmed up to 10 min before use, then the content of each test tube was transferred into a cuvette and the optical density of the solution in each test tube determined using a filter of 520 nm wave length.

Determination of white blood cell (WBC) count: The method of Coles (1986) was used for the white blood cells count. The white blood cell pipette was used to draw blood to 0.5 marks. The tip of the pipette was thereafter wiped and used to draw WBC diluting fluid to 11 marks above the bulb; the pipette was shaken thoroughly to mix the contents and then allowed to stand for 3 min. The counting chamber was charged with the diluting fluid after discarding the first few drops. One minute after charging the chamber the cells were counted with the help of light microscope at 40x objective. The cells in the four corners square were counted and multiplied by 1000 to give the total number of the cells counted in thousand per cubic millimeter ($\times 10^3 \text{mm}^3$).

Determination of differential leucocytic count (DLC): A dry micropipette was used to suck blood from the snipped part of the rats tail, a small drop of blood was applied to one end of a slide and quickly placed on the bench holding it in position, the end of the second slide was then placed in the drop and held there until the blood had spread across it. Blood is dried and stained with giemsa, washed with distilled water and allowed to dry for 2 min and then examined with microscope at low and high power magnification for cellular appearance (Osime *et al.*, 2004).

Statistical analysis: The results are presented as Mean \pm Standard deviation. Differences between means were assessed using Analysis of variance (ANOVA) and post test using Dunnett multiple comparison test (Mead and Curnow, 1982).

RESULTS

The quantitative phytochemical screening revealed the presence of low level of tannins, steroids and moderate level of saponins, carbohydrates, cardiac glycosides, flavonoids, Terpenes and Terpinoids (Table 1). The elemental analysis of the extract revealed the presence of calcium, magnesium, potassium, iron and

Table 1: Quantitative phytochemical analysis of the crude mesocarp extract of *Hyphaene thebaica* (doupalm)

Phytochemical constituents	Test	Inference
Tannins	Ferric chloride	+
	Fomaldehyde	+
	Chlorogenic acid	+
Saponins	1. Frothing	++
Alkaloids	Dragendorff's	-
	Mayer's	-
	Wagner's	-
	Molisch's	+
Carbohydrates	Barfoed's	-
	Combine reducing sugar	++
	Free reducing sugar	++
	Ketone's	+
	Pentoses	++
Phlobatannins	1. Hydrochloric acid	-
Cardiac glycosides	1. General test	++
Steroids	Lieberman's	+
	Salkowski's	+
Flavonoids	Shinoda's	++
	Ferric Chloride	+
Terpenes/Terpinoids	Lieberman- Buchar'ds	++
Anthraquinones	Salkowski's	++
	Free Anthraquinones	-

-, Absent, +: Low, ++: Moderate

Table 2: Elemental analysis of the crude mesocarp extract of *Hyphaene thebaica* (doupalm)

Elements	Concentration (ppm)	WHO standard conc. (mg dL ⁻¹ or ppm)
Calcium (Ca ²⁺)	263.9	360-800
Magnesium (Mg ²⁺)	11.66	-
Manganese (Mn ²⁺)	2.62	10-20
Copper (Cu ²⁺)	0.661	1-3
Zinc (Zn ²⁺)	8.44	15-20
Iron (Fe ³⁺)	18.62	0.5-50
Sodium (Na ⁺)	-	-
Potassium (K ⁺)	427.6	0.1-1
Nickle (Ni)	0.07	-
Silicon (Si)	2.72	-
Cobalt (Co)	0.04	-
Lead (Pb)	0.08	1-2
Molybdenum	0.104	-
Arsenic (As)	0.031	0.02-7

Table 3: Effect of crude mesocarp extract *Hyphaene thebaica* on white blood cells (WBC) (x10³ μL) count in wistar albino rats

Dosage (mg kg ⁻¹)	Period of Administration of aqueous pericarp extract of <i>H. thebaica</i> (weeks)			
	1	2	3	4
Control	8.3±0.18	8.4±0.16	8.4±0.11	8.5±0.08
400	8.5±0.11	13.1±0.41 ^b	14.1±0.47 ^b	14.7±0.19 ^b
600	8.7±0.13	13.5±0.37 ^b	14.4±0.16 ^b	15.0±0.18 ^b
800	8.6±0.15	13.7±0.13 ^b	14.6±0.17 ^b	16.0±0.08 ^b

X: Mean±SD, N: 5, ^bSignificant (p<0.05) increase as compared to control

sodium in moderate concentration, Manganese, zinc and silicon is low, whereas the amount of nickel, cobalt, molybdenum, arsenic and lead are negligible (Table 2). The crude mesocarp extract administered at the dosage of 400, 600 and 800 mg kg⁻¹ significantly (p<0.05) increased White blood cells count throughout the four weeks of administration (Table 3). During second, third and fourth week of administration, 600 and 800 mg kg⁻¹ of the extract significantly (p<0.05) increased Lymphocytic, Monocyte, Eosinophil and Basophil counts. Lymphocytes count is the parameter that significantly (p<0.05) increased on the administration of 400 mg kg⁻¹ of the extract at second and third week. The extract did not have effect on Neutrophils count throughout the period of treatment (Table 4).

DISCUSSION

The quantitative phytochemical screening of *Hyphaene thebaica* revealed the presence of low level of tannins, steroids and moderate level of saponins, carbohydrates, cardiac glycosides, flavonoids, Terpenes and Terpinoids. The product contains calcium, magnesium, potassium, iron and sodium in moderate concentration, Manganese, zinc and silicon are low in amount whereas nickel, cobalt, molybdenum, arsenic and lead very negligible (Table 1). Mineral elements such as Iron, copper and cobalt have been reported to stimulate bone marrow activity and enhance leucocyte production and maturation (Sumati and Kapoor, 1986). An increase in leucocyte levels was seen in rats exposed to 0.49 mg Ni/kg/day as nickel chloride in drinking water for 28 days (Weischer *et al.*, 1980). The presence of phytochemicals such as flavonoids, glycosides, reducing sugars and some elements such as Iron, Copper, Cobalt and Nickel in the extract could be the reason for the leucocytosis observed (Table 2). The significant increase in white blood cells caused by crude mesocarp extract of *Hyphaene thebaica* could be due stimulation of bone marrow stem cells to produce these cells which is an indication of immune-modulatory effect as was observed by other researchers exhibited by some plants (Adedapo *et al.*, 2007; Mohajeri *et al.*, 2007). The presence of phytochemicals such as glycosides and reducing sugars could be the reason for the leucocytosis (Ugochukwu *et al.*, 2003). Flavonoids protect both the hematopoietic committed stem cells and the formed blood cells from the attack of the reactive free radicals hence improving leucocytic production (Esmaili and Sonboli, 2010) (Table 3). The administration of 600 and 800 mg kg⁻¹ of the extract significantly (p<0.05) increased Lymphocytic, Monocytes, Eosinophils and Basophil counts. Lymphocytes count is the parameter that

Table 4: Effect of crude mesocarp extract of *Hyphaene thebaica* (doupalm) on differential leucocytic counts

Parameters	Dosage (mg kg ⁻¹)	Period of Administration (week)			
		1	2	3	4
Lymphocytes	Control	60.60±1.14	60.8±0.84	60.8±0.83	60.60±0.55
	400	61.80±0.84	65.0±1.14 ^b	67.20±1.30 ^b	71.60±0.55 ^b
	600	61.20±1.30	66.0±0.55 ^b	68.20±0.84 ^b	72.00±1.14 ^b
	800	61.00±1.58	66.8±0.45 ^b	68.40±0.55 ^b	74.00±0.00 ^b
Neutrophils	Control	31.40±1.14	30.60±0.68	30.60±1.52	21.40±0.89
	400	30.00±1.23	25.40±2.07	24.6±1.67	21.00±0.89
	600	30.60±1.67	23.20±1.64	23.6±1.14	18.20±1.92
	800	30.60±1.52	23.20±1.30	23.0±0.00	16.00±0.00
Monocytes	Control	4.20±0.45	4.60±0.55	4.60±0.55	4.60±0.55
	400	4.40±0.55	4.40±0.55	4.40±0.55	4.80±0.20
	600	4.40±0.55	5.80±0.45 ^b	5.40±0.55 ^b	5.80±0.20 ^b
	800	4.40±0.55	6.80±0.84 ^b	6.40±0.55 ^b	6.20±0.37 ^b
Eosinophils	Control	2.80±0.45	3.00±0.71	3.00±0.71	3.20±0.45
	400	2.80±0.45	3.00±0.71	2.80±0.45	4.20±0.45 ^b
	600	2.80±0.45	4.80±0.45 ^b	4.80±0.45 ^b	4.60±0.55 ^b
	800	3.00±0.00	4.20±0.84 ^b	4.00±0.00 ^b	4.40±0.55 ^b
Basophils	Control	0.00±0.45	1.00±0.00	1.20±0.45	1.20±0.45
	400	1.20±0.45	1.40±0.55	2.20±0.45	1.20±0.45
	600	1.00±0.00	1.40±0.55	4.00±0.00 ^b	3.00±0.00 ^b
	800	1.00±0.00	0.00±0.55	5.00±0.00 ^b	4.00±0.00 ^b

X: Mean±SD, N: 5, ^bSignificant (p<0.05) increase as compared to control

significantly (p<0.05) decreased on the administration of 400 mg kg⁻¹ of the extract at second and third week. The extract did not have effect on Neutrophils count throughout the period of treatment. The reduction in Neutrophils count may probably be due to cellular margination rather than destruction. The decrease in Neutrophils count did not affect the total white blood cell count (Table 4).

The above finding supports the folkloric application in Borno State of crude mesocarp extract of *Hyphaene thebaica* in improving immune status of convalescent or immunocompromised individuals.

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