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Full Length Research Paper

Effect of Methanolic Extract of the Fruit Pulp of *Hyphaene thebaica* (L) mart on some Heamatological parameters and Organ Histology in Rats

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ABSTRACT

The effects of methanol extract of *Hyphane thebaica* (L) mart fruit pulp on some haematological and histopathological parameters were investigated. Twenty albino rats of wistar strain were divided into four groups of five rats each. Group one serves as control while groups 2, 3 and 4 were administered daily orally by intubation with 200, 400 and 800mg/kg of 70% methanol extract of *H. thebaica* fruit pulp. All the rats were fed with normal diet (ECWA Mash, Jos, Nigeria) and water ad libitum for 28 days. Twenty-four hours after the last administration, the animals were sacrificed, heamoglobin concentration (Hb), packed cell volume (PCV), white blood cell (WBC), red blood cells (RBC), neutrophils and lymphocytes were assayed. Histology of liver, testes, lungs, kidney and spleen were carried out. Results revealed significant increase ($p < 0.05$) in the level of RBC, Hb and PCV. White blood cells- neutrophils and lymphocytes- were not affected. Toxic effects were seen in liver and testes at higher dose. It was concluded that the methanolic fruit pulp extract of *H. thebaica* is hematinic. However, its use at higher doses should be exercised with caution.

Keywords; *Hyphane thebaica*, heamatonic, organs histology

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INTRODUCTION

Plants have been part of human existence from creation and some plants have been known to be useful to our existence. These Medicinal plants have long been effective in the management of many diseases in many communities throughout the world and it also has a lot to offer in the treatment and prevention of many diseases such as anaemia, diabetes, etc (Beneskey and Gamble, 1993). There has been a surge in recent time in scientific investigations into folkloric claims on the medicinal importance of some plants.

Hyphaene thebaica (Common Names: Egyptian doum palm, gingerbread palm, duma; Fig.1) is a dioecious palm whose name is derived from the Greek word 'hyphaino' referring to the fibres from the leaves, which are used for weaving. native to Egypt, sub-Saharan Africa and West India. *Hyphaene thebaica* belongs to the family Palmae (Arecaceae) and subfamily Barassoideae (Burdkit, 1987). The plant is held in high esteem due to its economic importance, which often

makes its medicinal uses less expressed. For example, the powdered fruit is used as condiment to enhance flavor while the palms are often used for firewood and charcoal. The leaves are regarded in many communities as the most important, being useful as fuel in the dried form and provide the raw material used in basketry, making mats, brooms, coarse textiles, ropes, thatching and string. The root fibres are also useful in making fishing nets (Fletcher, 1997; Orwa *et al*, 2011).

In folkloric medicine, the fruit powder is commonly used in Kenya in making alcoholic drinks while the terminal meristem is tapped for making palm wine in other countries (NTBG 2012). The fruit pulp extract is also used in the treatment of bilharzias, bleeding especially after child birth and also as haematonic agent (Adaya *et al*; 1977; Von Maydell, 1986). The anti-cancer, antioxidant and antimicrobial activities of the fruit pulp has also been well documented (Carter, 1993; Hsu *et al*, 2006; Faten 2009; Dosunmu *et al*., 2006; Mohammed *et al*, 2010). When chewed, the fruit pulp is also used locally to control hypertension. The fruit pulp of

H. thebaica has been shown to contain nutritional trace minerals, proteins and fatty acids, in particular the nutritionally essential linoleic acid (Kamis *et al*, 2003) and many other beneficial nutrients (Abhosora *et al*, 2014) In a study, using aqueous suspension of the root of *Hyphaene thebaica* (L) Mart. Zanna *et al* (2008) reported that the extract was hyponatremic, hypocholesterolemic, hepato and nephrotoxic. In recent study, methanol extract of the fruit of *H. thebaica* (L) Mart reduced fasting hyperglycaemia (Shehu *et al*, 2014).

To date, there has not been any attempt at a scientific inquest into the folkloric claim that the fruit pulp of *H. thebaica* possess haematic activities. In this study, we investigated the effects of the methanol extract of the plant on some blood parameters and histology of some organs in rats.



Fig. 1
Egyptian doum palm
(<https://www.flickr.com/photos/3point141/8297211842>)

MATERIALS AND METHODS

Sample Collection and Identification

Fresh fruit of *Hyphaene thebaica*, was collected from Konduga local government area of Borno state, Nigeria. The plant was authenticated by a plant taxonomist with the Department of Biological Sciences, University of Maiduguri. The fruits were cleaned, debris removed, shade dried and ground into powder using mortar and pestle.

Extract Preparation

Hyphaene thebaica fruit pulp powder (500g) was macerated with one liter of 70% methanol in a glass jar for 2 days at room temperature. The extract was filtered, concentrated to dryness under reduced temperature and pressure on rotary evaporator. The percentage yield was calculated as 31.65%

Experimental Animals and Treatment

White Wistar strain Albino rats weighing between 120 and 150g were used for the study. The rats were obtained from the Animal house of the Veterinary pharmacology department, University of Maiduguri, Nigeria. They were maintained under standard condition of light (12-hour light). The rats were fed standard diet (growers mash, ECWA feed Nigeria Ltd) and

water *ad libitum*. Twenty Albino rats were divided into 4 groups of 5 rats each. Group 1 served as control while groups 2, 3 and 4 were administered daily orally by intubation at dose of 200, 400 and 800 mg/kg of 70% methanolic extract of *H. thebaica* fruit pulp for 28 days.

Haematological Analysis

Packed cell volume (PCV), White blood cell (WBC), Red blood cell (RBC), Haemoglobin (Hb), Total Leucocyte Count (TLC) and Differential Leucocyte Count (DLC) were estimated in the rats 24 hours before sacrifice. Packed Cell Volume (PCV) was estimated by the microhaematocrit method and Hb by the Drabskin method as described by Schalm *et al* (1975). Total leucocyte counts and Differential leucocyte counts were estimated by the method described by Schalm *et al* (1975).

Relative Organ Weights

Different organs heart, liver, lungs, spleen, kidney and testis were surgically dissected and weighed in grams (absolute organ weight). The Relative Organ Weights (ROW) of each animal were then calculated as follows:

$$\text{ROW:} = \frac{\text{Absolute weight (g)} \times 100}{\text{Body weight on the day of sacrifice}}$$

Histology

Tissue biopsies from kidney, liver, testis, heart and spleen were collected and fixed in 10% formalin solution. The tissues were dehydrated through graded concentration of ethanol (70%, 95% and absolute), cleared in xylene and embedded tissues were sectioned at 4-5 μm thickness and tissues were stained with Haematoxylin and Eosin (H & E) for light microscopic examination. Photomicrographs of section were taken using cyberpix (S-55IV) digital camera (Luna 1968)

Statistical analysis

The data obtained were presented as Mean and Standard error of mean (Mean \pm SEM). Differences among mean were analysed using analysis of variance (ANOVA), by computer statistical software graphpad instat® (2003). Probability value (p Value) ≤ 0.05 was considered significant

RESULTS

Acute toxicity

Methanolic extract of the fruit pulp was found to be nontoxic as it did not cause death even at the highest single oral dose of 5000mg/kg body weight. Hence the median lethal dose (LD50) was estimated to be $\geq 5000\text{mg/kg}$.

Haematological parameters

Table 1 shows the profile of blood parameters in rats following 28 days oral administration of different doses of methanolic extract of *H. thebaica* fruit. Results showed that Red blood cell (RBC) counts Haemoglobin (Hb) concentration and Packed Cell Volume were increased significantly for all the treatment groups, (P0.05). The neutrophils and lymphocytes of the treated groups were not affected when compared to the control group, (P>0.05).

Table 1

Effect of oral administration of different doses of *H. thebaica* fruit pulp methanolic extract on some haematological parameters (mean \pm SEM) in normal rats (n=5) for 28 days

Doses mg/kg	Hb (g/dl)	RBC ($\times 10^6$ /mm)	PCV (%)	WBC $\times 10^3$ /ml	Neut $\times 10^3$ /ml	Lymph $\times 10^3$ /ml
Control	10.20 \pm 1.20	3.50 \pm 0.06	39.60 \pm 1.36	5.70 \pm 0.92	19.20 \pm 0.37	63.20 \pm 0.20
200	13.76 \pm 1.33	4.01 \pm 0.09*	41.80 \pm 0.80	6.30 \pm 0.42	19.80 \pm 0.37	67.20 \pm 0.80
400	14.60 \pm 1.06	5.48 \pm 0.11*	44.40 \pm 1.36*	6.50 \pm 0.84	17.60 \pm 0.92	67.25 \pm 0.65
800	15.73 \pm 1.04*	6.64 \pm 0.08*	45.80 \pm 0.37*	7.40 \pm 0.97	18.34 \pm 0.33	66.33 \pm 1.01

Neut - Neutrophils, lymph – lymphocytes ; *P<0.05 significantly different from control

Table 2

Relative organs weight (g/100g body weight) of rats administered *H. thebaica* fruit methanolic extract for 28 days.

Doses mg/kg	Kidney	Liver	Lungs	Heart	Spleen	Testes
Control	0.76 \pm 0.40	4.08 \pm 0.13	0.78 \pm 0.16	0.34 \pm 0.03	0.92 \pm 0.12	1.62 \pm 0.12
200	0.73 \pm 0.40	3.78 \pm 0.08	0.73 \pm 0.04	0.35 \pm 0.02	0.73 \pm 0.08	1.54 \pm 0.15
400	0.74 \pm 0.30	3.76 \pm 0.04	0.67 \pm 0.06	0.36 \pm 0.02	0.67 \pm 0.11	1.24 \pm 0.05
800	0.75 \pm 0.10	3.60 \pm 0.07*	0.62 \pm 0.08	0.34 \pm 0.01	0.57 \pm 0.03	1.12 \pm 0.02*

* p<0.05 significantly different from control

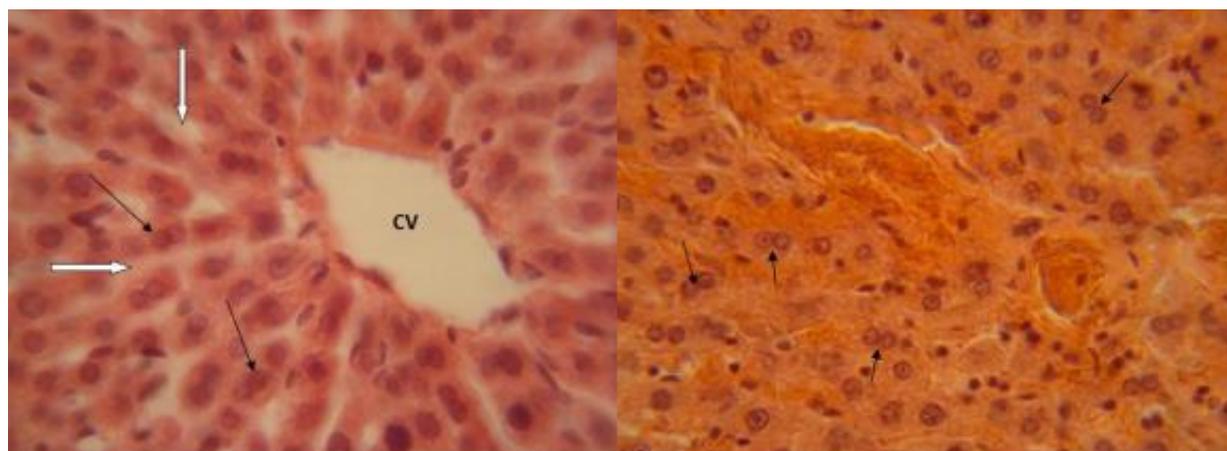


Plate 1:

(a) Photomicrograph of rat liver of control showing hepatocytes (black arrows) radiating away from the central vein (CV) and clear sinusoids (white arrows). No histopathological effect. H&E x400. (b) Photomicrograph of rat liver treated with 800mg/kg *H.thebaica* fruit pulp extract. Showing sinusoidal haemorrhage and multi-binuclear cells (arrows) H&E x400

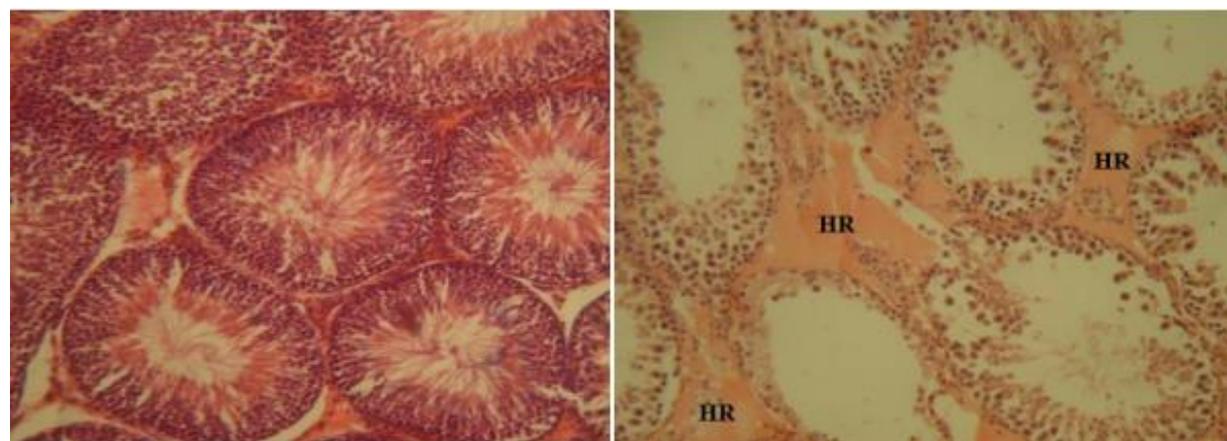


Plate 2:

(a) Photomicrograph of rat testis of control group showing normal features. No histopathological effect. H&E x200 (b) Photomicrograph of rat testis from rat treated with 800mg/kg of *H. thebaica* fruit pulp methanolic extract. Showing severe interstitial haemorrhage (HR) and eruption of the spermatogenic series H&E x200 extract.

Organ weights

Table 2 shows the effect of the extract on relative organ weights. There were no significant changes in relative weights of kidney, lungs, heart and spleen in all the treated groups ($P>0.05$) compared to the control group. However, in the 800mg/kg body weight group there was statistically significant decrease ($P<0.05$) in liver and testis weights compared with the control group.

Organ histology

The histopathological lesions were seen in some of the organs (kidney, spleen, lungs, liver, testes) of rats administered 800 mg/kg body weight. However, no observable pathologies were observed in the organs of those animals treated with lower doses (200 and 400 mg/kg) of the extract. The liver of rats treated with 800mg/kg showed sinusoidal haemorrhage and multi-binuclear cells (Plate 1). The testes showed severe interstitial haemorrhage, eruption of spermatogenic series (Plate 2)

DISCUSSION

Assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extract on blood related functions (Yakubu *et al*, 2007). In this study, prolonged administration of the extract significantly increased haemoglobin concentration, red blood cell count as well as packed cell volume compared to their respective control groups. While neutrophils and lymphocytes did not show any significant change compared to control. The improvement of RBC, Hb and PCV of treated animals is an indication of haematinic property of the extract. Substances that have hematinic effect are known to stimulate haematopoiesis by increasing production of RBC and improving the value of Hb and PCV (Barnard *et al*, 1989). Nwosu *et al*, (2008) reported that *H.thebaica* fruit pulp contains high amount of iron (47.90mg/100g) this may likely explain the haematinic potential of the fruit pulp of *H. thebaica*, that the extract has hematinic properties, is consistent with earlier report by Kamis *et al*, (2000); and Modu *et al*, (2000).

There were no significant changes in relative weight of kidney lungs, heart and spleen in all the treated groups ($P>0.05$) compared to the control group. However at 800mg/kg body weight group there was statistically significant decrease ($P<0.05$) in liver and testis compared with the control group. The histopathological lesion seen in some of the organs (liver and testes) were similar in group administered 800 mg/kg body weight. Shehu *et al* 2015 reported increase in the level of ALP and ALT at higher dose in the same extract. Increase in ALP is usually a characteristic of finding in obstructive hepatobiliary disease as found in cholesteric liver disease (Kaneko *et al* 1997).

The relative organ weight of testes showed a decrease in weight ($P<0.05$) and histology of the testes revealed severe interstitial haemorrhage and eruption of spermatogenic series in the highest dose (800mg/kg). According to Moore and Dally (1999) an increase in body organ weight ratio is an indication of inflammation while a reduction in the same

parameter can be adduced to cellular constriction, therefore this study suggests that the extract caused some constriction of testes. Helta *et al*, (2005) reported that chloroform extract of the fruit *H. thebaica* improved spermatic count of male rats at low concentration but could decrease the sperm count at high concentration.

The histology of kidney for 200, 400 and 800 mg/kg doses were normal. In a similar study using ethanolic fruit pulp extract of the plant, Kamis, *et al*, (2000), reported that at high concentration (1, 2.5 and 5g/kg) the plant extract is hypolipidemic, hepatotoxic and nephrotoxic. However, Modu *et al*, (2000) using aqueous pulp extract (1, 2.5 and 5g/kg) of *H.thebaica* found the extract to be hypolipidemic but nontoxic to both liver and kidney.

Medicinal plants such as *Aegel marmelos*, *Carissa congesta*, *Eugenia jambolana*, *Ficus carica*, *Pheonix sylvestris*, *Phyllanthus emblica*, *Vitus vinefera* and *Moringa oleifera* experimentally tried on rats have been found to significantly increase haematological parameters (Alada, 2000. Dina, 2000).

Although the methanolic extract of fruit pulp is heamatinic, and safe in rats treated with 200 and 400mg/kg body weight nonetheless it appears toxic at higher dose. It is recommended that other parts of the plant and different medium of extraction be tried to ascertain safety.

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