



Contents lists available at BioMedSciDirect Publications

International Journal of Biological & Medical Research

Journal homepage: www.biomedscidirect.com



Original Article

Haemopoietic activity and effect of Crude Fruit Extract of *Phoenix dactylifera* on Peripheral Blood Parameters.

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ARTICLE INFO

Keywords:

Aqueous Extract of *P. Dactylifera* (AEPD),
Methanolic Extract of *P. Dactylifera* (MEPD),
Haemopoietic,
Graded doses.

ABSTRACT

Aim: *Phoenix dactylifera* (date palm), has been reported to possess a variety of pharmacological activities which indicate its usefulness in various kinds of diseases and disorders. The present study was aimed at investigating the haemopoietic activity of crude fruit extract of *Phoenix dactylifera* (*P. dactylifera*) and its effect on peripheral blood parameters. **Method:** Fifty (50) Wistar rats weighing 100 to 200 grams and aged 2 to 3 months obtained from the Animal House of College of Medicine, University of Nigeria Enugu Campus were acclimatized for two weeks. They were divided into 10 groups of 5 rats per group labeled A1, A2, B1, B2, C1, C2, D1, D2, E1 and E2. The first 5 groups were orally administered with graded doses of the crude Aqueous Extract of *P. Dactylifera* (AEPD) (A1=0.4, B1=0.1, C1=0.52, D1=0.90 mg/kg body weight and E1 [control]=0.24ml Dimethylsulphoxide (DMSO) while the second 5 groups were orally administered with graded doses of the crude Methanolic Extract of *P. Dactylifera* (MEPD) (A2=0.4, B2=0.1, C2=0.52, D2=0.90 mg/kg body weight and E2[control]=0.24ml DMSO). The administrations were once daily for 112 days. On Day 113, 2.5ml of blood samples were collected from each rat through the retro bulbar plexus of the median canthus of the eye into tri-potassium- Ethylenediamine tetracetic acid (K3-EDTA) anticoagulant container and bone marrow was also collected for the analysis using standard operative procedure. **Results:** The results revealed dosage dependent significant increase in Absolute values, Red Blood Cell (RBC), Haemoglobin (Hb), Packed Cell Volume (PCV), Reticulocytes and Platelet count in both aqueous and methanolic extract when compared with the controls ($p < 0.05$, $p < 0.001$). The total and differential white blood cell counts and bone marrow examination did not differ significantly when compared with the controls ($p > 0.05$). **Conclusion:** The observed changes in this study has demonstrated that *P. Dactylifera* has haemopoietic effect when orally administered in Wistar rats.

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1. Introduction

The use of plant extracts for the treatment of ailments is on the increase especially with the recognition and incorporation of traditional medicine and food into the healthcare delivery system in Nigeria.[1] In Nigeria, traditional food and healing system play an important role in healthcare delivery and about 70-80% of the

population depends on traditional herbs for most of their ailments.[1] World Health Organization reported that expanding use of traditional and herbal medicine is gaining recognition globally.

Phoenix dactylifera (*P. Dactylifera*) fruit is a delicious desert fruit with much needed vitamins, minerals and energy to help one stay fit and healthy. It belongs to the family Arecaceae of the genus *Phoenix dactylifera*. The phytochemical constituents of *P. Dactylifera* include alkaloids, flavonoids, steroids, tannins, estereptens, carbohydrates, vitamins and phenolic acids.[2,3] *Phoenix dactylifera* also possesses numerous medicinal properties and is used in the treatment of anaemia, stroke, building up body weight, help in slowing ageing and in treatment of tooth ache.

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Despite the numerous health benefits of *P. dactylifera*, there is little or no information towards its haemopoietic activity and effects on peripheral blood parameters. This present study was designed to investigate the haemopoietic activities of crude fruit extract of *P. Dactylifera* and its effect on peripheral blood parameters. The variables to be measured include haemoglobin, haematocrit, red blood cell count, total and differential white blood cell count, platelet count, reticulocyte count, and bone marrow examinations.

2. Materials and Method

2.1. Collection of plant materials

The fruits of *P.dactylifera* were obtained from the Northern Nigeria and authenticated by a Taxonomist in the Department of Botany, University of Nigeria Nsukka and a voucher specimen was kept in the herbarium for future reference.

2.2. Animal housing

Fifty (50) Wistar rats were purchased and housed in the Animal House of the College of Medicine, University of Nigeria Enugu Campus, where they were allowed to acclimatize for two weeks. They were fed with commercially available rat feed and allowed access to the feed and water *ad libitum*.

2.3. Preparation of the plant extract:

2.3.1. Methanolic extract:

One hundred (100) grams of the fresh blended fruit of *P. dactylifera* were extracted exhaustively with 2.5 liters of methanol and the mixture sieved. The remaining methanol in the extract was evaporated to get the concentrated crude extract which was reconstituted with 3% Dimethylsulphoxide (DMSO) and stored in the refrigerator until needed.

2.3.2. Aqueous extract: One hundred (100) grams of the fresh blended fruit of *P. dactylifera* were extracted exhaustively with 200ml of water and the mixture sieved. The remaining water in the extract was evaporated to get the concentrated crude extract which was reconstituted with 3% Dimethylsulphoxide (DMSO) and stored in the refrigerator until needed.

2.4 Experimental Design

Fifty (50) Wistar rats weighing 100 to 200g and aged 2 to 3 months obtained from the Animal House of College of Medicine, University of Nigeria Enugu Campus were acclimatized for two weeks. They were divided into ten groups of five rats per group labeled A1, A2, B1, B2, C1, C2, D1, D2, E1 and E2. The first five groups were orally administered with graded doses of the crude aqueous extract of *P. dactylifera* (AEPD) [A1=0.4, B1=0.1, C1=0.52, D1=0.90mg/kg body weight and E1 [control]= 0.24ml DMSO] while the second five groups were orally administered with graded doses of the crude Methanolic Extract *P. dactylifera* (MEPD) [A2=0.4, B2=0.1, C2=0.52, D2=0.90mg/kg body weight and E2[control]=0.24ml DMSO]. The administrations were once daily for 112 days.

2.5 Sample Collection

On Day 113, 2.5ml of blood samples were collected from each rat through the retro bulbar plexus of the median canthus of the eye into K3-EDTA anticoagulant container for the analysis of peripheral blood parameters using standard operative procedure as described by Dacie and Lewis.[4] Bone marrow was also collected from some of the rats for cell count.

2.6 Statistical Analysis

The Statistical Package for Social Science (SPSS) computer software version 15 was used for data analysis. The results of the tests were analyzed using student's t-test at 95% confidence interval with p-value of 0.05 being considered as significant. The results were expressed as mean \pm standard deviation (\pm SD).

3. Results

The results of this study were expressed in tables 1, 2 and 3. Table 1 shows the mean and standard deviation of red blood cells (RBC), haemoglobin [Hb], haematocrit (PCV) and absolute indices of rats after oral administration of graded doses of AEPD and MEPD. Table 2 shows the mean and standard deviation of Total and differential white blood cell count of rats after oral administration of graded doses of AEPD and MEPD. Table 3 shows the mean and standard deviation of Platelets, Reticulocytes and Bone marrow count of rats after oral administration of graded doses of AEPD and MEPD. The results revealed dosage dependent significant increase in Absolute values, RBC, Hb, PCV, Reticulocytes and Platelet count in both aqueous and methanolic extract when compared with the controls ($p < 0.05$, $p < 0.001$). The total and differential white blood cell counts and bone marrow examination did not differ significantly when compared with the controls ($p > 0.05$).

4. Discussion

Phoenix dactylifera have been known to possess numerous medicinal properties and is used in the treatment of anaemia, stroke, building up body weight, help in slowing ageing and in treatment of tooth ache. Little or no haematological information has been recorded on *Phoenix dactylifera*. The aim of this study was to investigate the haemopoietic activities of crude methanolic and crude aqueous fruit extract of *P. dactylifera* and its effect on peripheral blood parameters in Wistar rats.

The results revealed dosage dependent significant increase in Absolute values, red blood cells (RBC), Haemoglobin (Hb), Haematocrit (PCV), Reticulocytes and Platelet count in both aqueous and methanolic extract when compared with the controls ($p < 0.05$, $p < 0.001$). The total and differential white blood cell counts and bone marrow examination did not differ significantly when compared with the controls ($p > 0.05$).

Table 1. Mean \pm standard deviation of RBC, Hb, PCV and absolute indices of rats after oral administration of graded doses of AEPD and MEPD.

Group	RBC (X10 ¹² /L)	Hb (g/dl)	PCV (%)	MCHC (g/dl)	MCV (fL)	MCH (Pg)
A1 (0.4mg/kg) AEPD	8.18 \pm 0.11	11.7 \pm 0.11 ^{ab}	48.2 \pm 0.43	24.34 \pm 0.05	59.1 \pm 0.91	59.1 \pm 0.91
B1 (0.1mg/kg) AEPDC	7.00 \pm 0.83	12.2 \pm 0.31 ^c	45.6 \pm 2.53	27.1 \pm 2.62	67.5 \pm 5.23	67.5 \pm 5.23
1 (0.52mg/kg) AEPDD	6.75 \pm 0.85	13.8 \pm 0.21 ^c	48.4 \pm 2.78	28.9 \pm 1.64	74.0 \pm 4.61	74.0 \pm 4.61
1 (0.90mg/kg) AEPDE	6.10 \pm 0.86	13.4 \pm 0.10 ^c	45.1 \pm 2.81	29.2 \pm 1.51	77.0 \pm 4.53	77.0 \pm 4.53
1(0.24ml) % DMSO	5.64 \pm 0.56	9.38 \pm 0.53	35.4 \pm 2.20	26.4 \pm 0.94	64.1 \pm 3.88	64.1 \pm 3.88
ControlA2 (0.4mg/kg)	8.11 \pm 0.11	11.5 \pm 0.13	47.9 \pm 1.10	23.2 \pm 0.51	59.5 \pm 1.02	59.5 \pm 1.02
MEPDB2 (0.1mg/kg)	4.82 \pm 0.15	12.3 \pm 0.10 ^L	39.4 \pm 0.71	31.0 \pm 0.50	82.6 \pm 1.85	82.6 \pm 1.85
MEPDC2 (0.52mg/kg)	5.63 \pm 0.61	15.1 \pm 0.31 ^L	46.6 \pm 2.60	31.1 \pm 1.31	82.3 \pm 3.74	82.3 \pm 3.74
MEPDD2 (0.90mg/kg)	6.17 \pm 0.52	13.3 \pm 0.13 ^L	44.4 \pm 2.94	30.0 \pm 1.55	77.9 \pm 4.98	77.9 \pm 4.98
MEPDE2 (0.24ml) %	6.15 \pm 0.52	8.7 \pm 0.40	36.3 \pm 2.62	25.5 \pm 0.67	59.8 \pm 1.5	59.8 \pm 1.5
DMSO Control						
F-ratio	2.620	60.307	4.762	6.063	6.979	16.979
p-value	P<0.05*	P<0.001*	P<0.001*	P<0.001*	P<0.001*	P<0.001*

Where: AEPD= Aqueous Extract of *P. Dactylifera*; MEPD= Methanol Extract of *P. Dactylifera*

*= Statistically significant.

Table 2. Mean \pm standard deviation of Total and differential white blood cell count of rats after oral administration of graded doses of AEPD and MEPD.

Group	TWBC (X10 ⁹ /L)	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)
A1 (0.4mg/kg)	8.62 \pm 1.02	51.8 \pm 3.98	46.8 \pm 3.92	0.40 \pm 0.24	0.60 \pm 0.24	0.00 \pm 0.00
AEPDB1 (0.1mg/kg)	6.21 \pm 1.23	47.2 \pm 2.78	50.6 \pm 2.77	0.60 \pm 0.40	1.40 \pm 0.40	0.20 \pm 0.20
AEPDC1 (0.52mg/kg)	11.20 \pm 2.7	55.2 \pm 2.77	41.4 \pm 2.87	1.60 \pm 0.24	1.80 \pm 0.37	0.00 \pm 0.00
AEPDD1 (0.90mg/kg)	78.32 \pm 2.68	55.2 \pm 3.87	42.8 \pm 4.02	1.40 \pm 0.24	0.20 \pm 0.20	0.20 \pm 0.20
AEPDE1 (0.24ml) 5%	6.12 \pm 1.13	51.2 \pm 3.51	46.2 \pm 3.14	1.40 \pm 0.40	0.80 \pm 0.20	0.04 \pm 0.40
DMSO ControlA2 (0.4mg/kg)	7.62 \pm 0.46	55.2 \pm 2.49	42.2 \pm 2.37	1.40 \pm 0.46	0.80 \pm 0.40	0.60 \pm 0.24
MEPDB2 (0.1mg/kg)	5.94 \pm 0.51	55.4 \pm 1.77	46.2 \pm 3.43	1.40 \pm 0.40	1.40 \pm 0.50	0.00 \pm 0.00
MEPDC2 (0.52mg/kg)	4.92 \pm 0.24	49.0 \pm 1.78	48.4 \pm 2.14	1.25 \pm 0.25	1.00 \pm 0.44	0.40 \pm 0.24
MEPDD2 (0.90mg/kg)	9.28 \pm 1.97	52.2 \pm 2.03	44.6 \pm 2.13	1.60 \pm 0.37	1.40 \pm 0.40	0.20 \pm 0.20
MEPDE2 (0.24ml) %	7.04 \pm 0.84	44.4 \pm 2.90	53.2 \pm 3.10	0.60 \pm 0.40	1.40 \pm 0.46	0.00 \pm 0.00
DMSO Control	1.482	1.760	1.501	1.955	1.807	1.111
F-ratio	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
p-value						

Where: AEPD= Aqueous Extract of *P. Dactylifera*; MEPD= Methanol Extract of *P. Dactylifera*

*= Statistically significant.

Table 3. Mean \pm standard deviation of Platelets, Reticulocytes and Bone marrow count of rats after oral administration of graded doses of AEPD and MEPD.

Group	Platelet (X10 ⁹ /L)	Reticulocyte (%)	Bone marrow count (x10 ⁶ /femur)
A1 (0.4mg/kg) AEPD	620.80 \pm 18.20	0.98 \pm 0.13	1.50 \pm 0.53
B1 (0.1mg/kg) AEPD	284.80 \pm 54.42	1.90 \pm 0.13	-
C1 (0.52mg/kg) AEPD	259.20 \pm 10.74	2.90 \pm 0.24	-
D1 (0.90mg/kg) AEPD	277.80 \pm 32.72	2.60 \pm 0.10	0.80 \pm 0.05
E1 (0.24ml) 5% DMSO	134.40 \pm 28.45	1.00 \pm 0.16	1.40 \pm 0.40
Control	415.40 \pm 73.36	1.30 \pm 0.12	1.40 \pm 0.42
A2 (0.4mg/kg) MEPD	265.80 \pm 51.91	1.90 \pm 0.20	-
B2 (0.1mg/kg) MEPD	295.00 \pm 3.49	3.02 \pm 0.20	-
C2 (0.52mg/kg) MEPD	347.20 \pm 10.33	2.76 \pm 0.11	1.15 \pm 0.15
D2 (0.90mg/kd) MEPD	104.80 \pm 22.21	1.30 \pm 0.12	2.00 \pm 0.00
E2 (0.24ml) % DMSO	14.949	28.802	1.696
Control	P<0.001*	P<0.001*	p>0.05
F-ratio			
p-value			

Where: AEPD= Aqueous Extract of *P. Dactylifera*; MEPD= Methanol Extract of *P. Dactylifera*

*= Statistically significant.

The phytochemical constituents of *Phoenix dactylifera* include alkaloids, flavonoids, steroids, tannins, estertepens, carbohydrates, vitamins and phenolic acids.[2,3] This result pattern has indicated that both crude methanolic and crude aqueous fruit extract of *P.dactylifera* may have stimulatory effect on the bone marrow for the haemopoietic activities. The observed effect may be as a result of the tannin, ascorbic acid [5] and phenol contents of *P. dactylifera*.[6]

This extract has been reported to be an anti-microbial agent, anti-inflammatory, anti-ulcer, and analgesic.[7] It has also been reported that *P. dactylifera* is effective in the treatment of gonorrhoea.[8] This may probably be as a result of its leucocytic activity and the blood film also revealed mild to moderate leucocytosis with lymphocyte as the predominant leukocyte. The observed significant increase in total white blood cell count agrees with the findings of previous researchers that it has anti microbial activity.[9] The observed significant increase in Hb and PCV agrees with the earlier reports that this extract possess anti anaemic properties. The observed reticulocytosis in this study shows that the extract supports haemopoiesis. Platelet count was also increased showing that this extract can be used to correct bleeding disorder as a result of thrombocytopenia.

5. Conclusion

The present study has demonstrated that the crude methanolic and aqueous fruit extracts of *P. dactylifera* may possess properties capable of supporting increased erythropoietin synthesis by the liver to stimulate the bone marrow to produce more cells (haemopoiesis) and cause the observed effects on peripheral blood parameters in Wistar rats.

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