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Effect of Methanol and Ethanol Extracts of Raphia Vinifera Fruit Mesocarp on the Hematological Parameters of Wistar Albino Rats

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ABSTRACT

Hematology refers to the study of the numbers and morphology of the cellular elements of the blood; its studies are useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood. *Raphia vinifera* have been employed in the management and treatment of various ailments. This study aimed at determining the effect of methanol and ethanol extracts of *Raphia vinifera* fruit mesocarp on the hematological parameters of wistar albino rats. Twenty one (21) healthy male wistar rats (166-274g) were randomly assigned into three groups (n=3) thus: control, methanol and ethanol groups with 50mg/kg, 100mg/kg and 1000mg/kg dosages. Both methanol and ethanol groups were treated with daily oral administration of 0.1ml of the extracts for 14 days. Blood samples were collected from each animal through cardiac puncture for hematological analysis. In red blood cell indices, the mean values of RBC, HGB, HCT, RDW-CV and RDW-SD of both methanol and ethanol extracts shows higher values than the control. Meanwhile, MCV, MCH and MCHC of the ethanol extract increased when compared to control. White blood cell indices result shows higher values of GRA in methanol and ethanol extracts compared to the control, and WBC, LYM and MON shows increase in 1000mg/kg of ethanol extracts compared to control. In platelet indices, there was an increase in methanol and ethanol extracts of the plant comparing with the control on PLT count, PCT, PDW. Also, MPV and P-LCR exhibited higher counts of 100mg/kg ethanol extract. In conclusion, methanol and ethanol extracts of *Raphia vinifera* fruit mesocarp shows increased in red blood cell and platelet indices counts while ethanol extract shows increased in white blood cell indices counts compared to the control. In methanol extracts 50mg/kg dose exhibited better count than other doses, while ethanol extracts, 50mg/kg exhibited better counts in RBC indices, 1000mg/kg in WBC and 100mg/kg in platelet indices. In comparison, ethanol extract exhibited better counts than methanol extract. We therefore recommend that *R. vinifera* fruit mesocarp should be consumed in moderate quantities for hematological benefits.

Introduction

Several plants have been employed therapeutically for the management and treatment of several ailments. The plant, *Raphia vinifera* is an example of such plants. *Raphia vinifera* also called Bamboo palm is a medicinal and nutritional plant particularly abundant in swamps and other moist locations of the edges of creeks of Niger Delta and other Deltaic States in Nigeria, and distributed from Benin eastward to the Democratic Republic of Congo [1]. It is a solitary and Hapazanthic palm tree with unbranched, rather stout stem that is 5m tall belonging to the family "Arecaceae" [2].

Raphia vinifera, commonly known as king bamboo palm is an evergreen, single-stemmed palm tree. The medicinal property of different parts of the plant has been demonstrated, preparation of the root are used against toothache. Fibers taken from the leaf sheath are taken against digestive disorders, and fermented sap from the inflorescence serves as a laxative. A decoction of the fruit pulp is used as a treatment for dysentery, and an infusion of fruit staunches hemorrhaging. In West Cameroon, a decoction of the apical bud of *R. vinifera* is used for the treatment of gonorrhea and other genito-urinary infections, while the leaf is used against witchcraft, poison, and various sexually transmitted diseases [3]. The oily mesocarp of fruits is eaten and can be fermented into a strong drink for ritual use [3] or to increase sexual ability as per the traditional medicine. The impressive range of medicinal uses of *R. vinifera* fruits may be due to the presence of various phyto-constituents such as alkaloids, flavonoids, saponins, and tannins [4].

Hematology refers to the study of the numbers and morphology of the cellular elements of the blood; its studies are useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood [5] [6]. Blood which is a vital special circulatory tissue is composed of cells suspended in a fluid intercellular substance (plasma) with the major function of maintaining homeostasis[7]. Hematological components, which consist of red blood cells, white blood cells and Platelet indices concentration, are valuable in monitoring feed toxicity especially with feed

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constituents that affect the blood as well as the health status of animals and humans [8].

Some hematological components adopted in this study includes the Red blood cell indices: Red blood cell (RBC) count, Hemoglobin (HGB), Hematocrit (HCT), Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), Red cell distribution width coefficient of variation (RDW-CV) and Red cell distribution width standard division (RDW-SD). White blood cell indices: White blood cell (WBC) count, Lymphocytes (LYM), Monocyte (MON) and Granulocytes (GRA). Platelet indices: Platelet blood test (PLT), Mean platelet volume (MPV), Plateletcrit (PCT), Platelet distribution width (PDW) and Platelet large cell ratio (P-LCR).

It is worthy of note that evident studies are already available that almost all parts of the plant are of economic importance, and some parts like the fruits is on nutritional value to man, but to date, no study has compared the effect of the methanol and ethanol extracts of its fruit mesocarp in the hematological parameters of animals, hence this study.

Materials and Methods

Chemicals/reagents used

All chemicals and reagents used were of analytical standard. They include distilled water, methanol, ethanol and chloroform.

Sample collection

The fruits of *Raphia vinifera* were gotten from the *Raphia* tree in Federal Polytechnic, Ekowe, Bayelsa State, Nigeria. The hard exocarps were broken to get the edible mesocarp and were thoroughly washed and sun-dried for two days. The mesocarp was oven dried and its net weight of 248g was taken using weighing balance. The dry *Raphia vinifera* mesocarp was then pulverized to powder form using an electric blender.

Preparation of plant extracts

Preparation of methanol extract

124g of dried grinded Raphia vinifera fruit mesocarp was accurately measured using a weighing balance and placed into a suitable container, after which 1000mls of methanol was added to the container and kept for 72 hours then a mesh was used to macerate the extract to get a liquid concentrate. The liquid concentrate was then transferred into a water bath system for the methanol to evaporate leaving behind the solid powdered concentrate which weighed 50.6 grams at a stock concentration of 100mg/ml.

Preparation of ethanol extract

122g of dried grinded Raphia vinifera fruit mesocarp was accurately measured using a weighing balance and placed into a suitable container, after which 1000mls of ethanol was added to the container and kept for 72 hours then a mesh was used to macerate the extract to get a liquid concentrate. The liquid concentrate was then transferred into a water bath system for the methanol to evaporate leaving behind the solid powdered concentrate which weighed 43.7 grams at a stock concentration of 100mg/ml.

Grouping of experimental animals

Twenty one (21) healthy male wistar albino rats of about three (3) months weighing 166 – 274 grams were used in this study. They were purchased from the animal house of Science Laboratory Technology, University of Benin. They were assigned by randomization into 3 groups (n=3) thus: control, 50mg/kg, 100mg/kg and 1000mg/kg extracts of methanol and ethanol groups. Methanol and ethanol groups received rat feed and extracts and control group received feeds and normal saline once daily. All treatments lasted for 14 days.

Collection of blood samples

Blood samples were collected directly from the interior (the heart) of the experimental animals after being sacrificed. All animals were anesthetized with chloroform for easy dissection and a clean syringe of 5ml was used to collect blood from the rats into pre-labelled Ethylenediaminetetracetate (EDTA) sample bottles. Blood samples were collected and were taken to the medical laboratory for hematological tests.

Hematological analysis

Hematological parameters were analyzed using automated cell counter (Coulter Electronics, Luton, Bedfordshine, UK) having standard calibrations in line with the instructions of the manufacturer at Medical Laboratory Department of University of Benin Teaching Hospital (UBTH), Edo State. Parameters measured were: Red blood cell indices: Red blood cell (RBC) count, Hemoglobin (HGB), Hematocrit (HCT), Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), Red cell distribution width coefficient of variation (RDW-CV) and Red cell distribution width standard division (RDW-SD). White blood cell indices: White blood cell (WBC) count, Lymphocytes (LYM), Monocyte (MON) and Granulocytes (GRA). Platelet indices: Platelet blood test (PLT), Mean platelet volume (MPV), Plateletcrit (PCT), Platelet distribution width (PDW) and Platelet large cell ratio (P-LCR).

Results

Table 1.Body Weight of Animal with Methanol Extract (*Mean ± SEM, n = 3)*

	Control	50mg/kg	100mg/kg	1000mg/kg
Day 1	236±21.4	227±6.65	186±7.17	228±31.3
Day 8	198±4.73	230±3.84	200±10.9	262±6.36
Day 14	182±0.88	214±2.33	183±8.09	157±4.91

Table 2. Red Blood Cell Indices of Methanol Extract (*Mean ± SEM, n = 3)*

Parameters	Control	Methanol Extract		
		50mg/kg	100mg/kg	1000mg/kg
RBC (10^6/µl)	6.68±0.35	6.91±0.35	7.09±0.16	7.42±0.29
HGB (g/dL)	14.1±0.35	15.2±0.26	15.3±0.25	16.2±0.41
HCT (%)	41.5±0.58	42.6±0.58	43.2±0.55	44.2±1.68
MCV (μm^3)	63.2±2.29	61.7±1.22	61.0±2.08	59.6±0.48
MCH (Pg)	21.7±0.53	22.0±0.47	21.6±0.26	21.8±0.35
MCHC (g/dL)	34.4±0.38	35.7±1.07	35.4±0.92	34.0±3.49
RDW-CV (%)	17.8±0.72	18.37±0.26	17.9±0.49	18.3±0.91
RDW-SD (μm^3)	38.9±0.69	39.1±2.28	36.5±2.26	37.0±1.61

Note: RBC- Red blood cell, HGB- Hemoglobin, HCT-Hematocrit, MCV-Mean corpuscular volume, MCH- Mean corpuscular hemoglobin, MCHC-mean corpuscular hemoglobin concentration, RDW-CV -Red cell distribution width coefficient of variation, RDW-DS - Red cell distribution width standard division, µl-microliter, μm^3-micrometer cube, Pg- petagram, g/dl-grams per deciliter, %- percentage

Table 3. White Blood Cell Indices of Methanol Extract (*Mean ± SEM, n = 3)*

Parameter	Control	Methanol extract		
		50mg/kg	100mg/kg	1000mg/kg
WBC (10^3/µL)	5.17±0.60	3.56±0.52	3.06±0.50	2.46±0.66
LYM (%)	73.5±3.17	58.3±1.12	70.6±4.48	65.0±4.03
MON (%)	16.5±2.31	21.5±0.60	16.1±1.57	19.5±2.34
GRA (%)	8.40±0.61	20.2±1.71	13.3±2.99	15.5±2.74

Note: WBC- White blood cell, LYM- Lymphocytes, MON- Monocyte, GRA- Granulocytes, %-percentage, µl-microliter

Table 4. Platelet Indices of Methanol Extract (*Mean ± SEM, n = 3)*.

Parameter	Control	Methanol Extract		
		50mg/kg	100mg/kg	1000mg/kg
PLT (10^3/µL)	845±30.3	967±51.9	863±38.9	4322±3412.2
MPV (μm^3)	7.80±0.36	7.50±0.20	7.10±0.06	7.16±0.32
PCT (%)	0.68±0.28	0.72±0.05	0.61±0.22	0.70±0.05
PDW (%)	10.4±4.98	15.1±0.20	14.4±0.41	15.7±0.70
PLCR (%)	10.0±2.17	8.33±0.85	6.50±0.17	7.16±1.64

Note: PLT-Platelet blood test, MPV-Mean platelet volume, PCT-Plateletcrit, PDW-Platelet distribution width, P-LCR- Platelet large cell ratio, µl-microliter, μm^3-micrometer cube

Table 5. Body Weight of Animals with Ethanol Extract (*Mean ± SEM, n = 3)*.

	Control	50mg/kg	100mg/kg	1000mg/kg
Day 1	236±21.4	189±10.9	188±1.45	180±5.46
Day 8	198±4.73	195±8.99	197±2.65	198±9.17
Day 14	182±0.88	182±1.45	190±3.05	182±6.00

Table 6: Red Blood Cell Indices of Ethanol Extract (*Mean ± SEM, n = 3)*

Parameters	Control	Ethanol Extract		
		50mg/kg	100mg/kg	1000mg/kg
RBC (10^6/µl)	6.68±0.35	7.3±0.26	6.73±0.50	6.77±0.02
HGB (g/dL)	14.1±0.35	16.0±0.40	15.3±0.87	15.0±0.33
HCT (%)	41.5±0.58	46.2±1.00	43.6±1.84	43.2±1.04
MCV (μm^3)	63.2±2.29	63.4±1.41	65.2±2.68	63.8±1.73
MCH (Pg)	21.7±0.53	22.0±0.58	22.4±0.40	22.2±0.56
MCHC (g/dL)	34.4±0.38	34.6±0.21	34.4±0.80	34.7±0.13
RDW-CV (%)	17.8±0.72	19.7±0.65	18.5±0.79	19.3±1.27
RDW-SD (μm^3)	38.9±0.69	42.8±0.53	40.6±2.42	42.4±1.27

Note: RBC- Red blood cell, HGB- Hemoglobin, HCT-Hematocrit, MCV-Mean corpuscular volume, MCH- Mean corpuscular hemoglobin, MCHC-mean corpuscular hemoglobin concentration, RDW-CV -Red cell distribution width coefficient of variation, RDW-SD - Red cell distribution width standard division, µl-microliter, μm^3-micrometer cube, Pg- petagram, g/dl-grams per deciliter,%-percentage.

Table 7. White Blood Cell Indices of Ethanol extract (*Mean ± SEM, n = 3.)*

Parameter	Control	Ethanol Extract		
		50mg/kg	100mg/kg	1000mg/kg
WBC (10^3/µL)	5.17±0.60	3.73±0.36	4.10±0.72	5.53±0.44
LYM (%)	73.5±3.17	66.6±3.11	77.8±3.95	68.5±3.48
MON (%)	16.5±2.31	18.4±0.37	14.6±2.35	16.7±1.36
GRA (%)	8.40±0.61	15.0±3.47	7.53±1.62	14.8±2.13

Note: WBC- White blood cell, LYM- Lymphocytes, MON- Monocyte, GRA- Granulocytes, %- percentage, µL- microliter

Table 8. Platelet Indices of Ethanol Extract (*Mean ± SEM, n = 3)*

Parameter	Control	Ethanol Extract		
		50mg/kg	100mg/kg	1000mg/kg
PLT (10^3/µL)	845±30.3	948±132.5	1009±243.1	827±31.8
MPV (μm^3)	7.80±0.36	7.20±0.20	7.90±0.31	7.13±0.13
PCT (%)	0.68±0.28	0.67±0.78	0.80±0.21	0.58±0.28
PDW (%)	10.4±4.98	15.5±0.36	14.9±0.59	15.2±0.73
PLCR (%)	10.0±2.17	6.86±0.98	10.9±1.76	7.10±0.78

Note: PLT-Platelet blood test, MPV-Mean platelet volume, PCT-Plateletcrit, PDW-Platelet distribution width, P-LCR- Platelet large cell ratio, %- percentage, µL- microliter, µm-micrometer

Discussion

Blood which is a vital special circulatory tissue is composed of cells suspended in a fluid intercellular substance with the major function of maintaining homeostasis [7]. Hematological components, which consist of red blood cells, white blood cells and platelet indices concentration, are valuable in monitoring feed toxicity especially with feed constituents that affect the blood as well as the health status of animals and humans [8].

Red blood cells (RBCs) serve as a carrier of hemoglobin. It is this hemoglobin that reacts with oxygen carried in the blood to form oxy-hemoglobin during respiration [9]. Red blood cell indices like RBC count, HGB, HCT, MCV, MCH, MCHC, RDW-SD and RDW-CV were analyzed (Table 2 & 6), and the mean values of RBC, HGB, HCT, RDW-CV and RDW-SD of both methanol and ethanol extracts shows higher values than the control. Meanwhile, MCV, MCH and MCHC of the ethanol extract were higher than the control. This result is in agreement with that of "[7]".

A similar work by "[10]" reported a significant increase in RBC count following administration of aqueous extract of similar plant which is in agreement with the result. Our results suggest that methanol extract of *R. vinifera* fruit mesocarp at the administered doses did not affect MCV, MCH and MCHC and oxygen-carrying capacity of blood. From the present result differentiation of precursor cells and maturation of red blood cells (RBCs) were affected by the extracts because there was variation in the sizes of circulating RBCs as indicated by the change in RDW-SD and RDW-CV. According to "[7]" red blood cell is involved in the transport of oxygen and carbon dioxide in the body, which are its primary function in the human body. However, Ethanol extract of *R. vinifera* fruits mesocarp extracts exhibited better result than methanol and 50mg/kg dosage was also better in red blood cell indices.

White blood cells (WBCs) are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders and microbial activities. White blood cell indices; WBC count, LYM, MON and GRA were analyzed (Table 3 & 7) and the result shows higher values of GRA both Methanol and Ethanol extracts compared to the control. The increase in WBC and lymphocytes counts in the extracts group is in tandem with "[10]". The increase in TWBC and lymphocytes counts indicates the activation of the defence mechanism and immune system of the rats. Increase in lymphocytes count (lymphocytosis) occurs during infections. Certain cytotoxic substances (such as alkaloids and saponins) are present in the extract of *Tetrapleura tetraptera* [11]. The significant increase in TWBC and lymphocyte count in aqueous group compared with control which does not occur in the ethanol group is probably due to difference in the doses administered (60mg/kg for aqueous and 40mg/kg for ethanol) implying that more cytotoxic components were present in the aqueous extract than in the ethanol extract used. Further work is needed for more clarification. It is possible that the increase in TWBC and lymphocyte counts is a consequence of the activation of the immune system in a bid to protect the body of the rats from attack by the cytotoxic components of the aqueous extract. While WBC, LYM and MON shows increase in 1000mg/kg of ethanol extracts compared to control. The result is in agreement with works of "[11]".

Platelet indices are potentially useful markers for the early diagnosis of thromboembolic diseases. MPV and PDW help indiscriminating causes of thrombocytopenia as hypo-productive of hyper-destructive like immune thrombocytopenic purpura. Platelet indices, PLT count, MPV, PCT, PDW and P-LCR were analyzed, and there was an increase in methanol and ethanol extracts of the plant when compared with the control on PLT count, PCT, PDW. While MPV and P-LCR had higher values of 100mg/kg ethanol extract compared to the control as shown in Tables 3 and 8. This finding is in agreement with the report by "[10]" where aqueous extract of TTE was reported to significantly increase platelet count. The non-significant change in MPV and P-LCR from the results indicates that methanol and 50mg/kg and 1000mg/kg dosage of ethanol fruit extracts of *R. vinifera* probably did not affect the role of platelets (i.e. aggregation and formation of platelet plug) in the blood clotting process. Platelet aggregation is not affected when P-LCR is not affected significantly.

Conclusion

In conclusion, methanol and ethanol extracts of *Raphia vinifera* fruit mesocarp shows increased in red blood cell and platelet indices counts while ethanol extract shows increased in white blood cell indices counts when compared with control. In methanol extracts 50mg/kg dose exhibited better count than other doses, while ethanol extracts, 50mg/kg exhibited better counts in RBC indices, 1000mg/kg in WBC and 100mg/kg in platelet indices. In comparison, ethanol extracts exhibited better counts than methanol extract. We therefore recommend that *R. vinifera* fruit mesocarp should be consumed in moderate quantities for hematological benefits.

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