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Comparative Hematological Effect of Seed and Stem Bark Extracts of *Carapa procera* D.C Meliaceae in Male Rodents

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ABSTRACT

Background: Blood system plays a vital role in the survival of mammals. *Carapa procera* is a medicinal plant which the seed is used in treatment of malaria while stem bark is used for the management of male erectile dysfunction in folkloric medicine. **Objective:** Evaluation of the hematological parameter is a tool to assess toxicity and to corroborate the safety or otherwise of this medicinal plant in humans. **Methods:** The seed and stem bark were collected from the wild and extracted using cold maceration and LD₅₀ were determined using Lorke's method. The extracts and fractions were administered at a dose of 86.60, 173.21, 259.81 for crude seed extract, while fraction was administered at a dose of 173.21 mg/kg/day while the crude stem bark was administered at 44.72, 89.44, 134.16 mg/kg/day while the median dose was administered daily for all the fractions for 7 days. On the 8th day the rats were anaesthetized and blood sample collected via cardiac puncture. **Results:** The results of the assessment showed that the seed extract significantly suppresses the lymphocytes at p<0.05-0.001 when compared to control, while the stem bark showed insignificant effect on the lymphocytes. However, the stem bark extract increased the level of hematocrit which could be due to dehydration and may lead to anemia. **Conclusion:** The seed and stem bark extracts affect hematological parameters such as suppression of the lymphocytes and elevation of the hematocrit level in humans using this medicinal plant.

Keywords: *Carapa procera*, Lymphocyte, Hematological indices, Anemia.

INTRODUCTION

The use of medicinal plants is as old as human civilization. The blood system transport essential nutrients to various organs and tissues. The transportation of oxygen to various organs and prevention of the body from invading microorganisms depend on the hematological system which play an essential role on the immune system. Also, investigation of the possible effects of phytochemicals on the hematological indices in animals is also an important tool in toxicity assessment^[1-3]. This study was carried out to determine the effect of *Carapa procera* stem bark and seed extracts on the hematological parameters in male rats since the stem bark is used in the management of erectile dysfunction in men whereas the seed is used in the treatment of malaria by the I jaw in Niger Delta region of Nigeria^[4].

MATERIALS AND METHODS

Materials

All reagents and Materials are bought from reputable pharmaceutical companies.

Collection and Identification

The plant was collected and identified as stated by Owaba *et al.*, (2021)^[4].

Extraction

Seed Extraction

The dried powdered seed weighing 1505 g was extracted via successive maceration at room temperature using 5 L each of the following's solvents; n-Hexane, dichloromethane and 70% methanol for 7-days respectively. Each of the extract was concentrated at 50°C *in vacuo*. The crude extract was obtained by extracting about 352 g of the seed with 2.0 L of 70% methanol and filtered daily for 7 days and the extract concentrated *in vacuo*.

Stem bark Extraction

About 3271 g of the powdered stem bark was extracted successively using n-hexane, dichloromethane and 70% methanol (4x2.5L) respectively, for 7 days. The crude extract about 490 g of the powdered stem bark was extracted with 2.5 L of 70% methanol filtered daily and a fresh solvent added for 7 days. The extracts concentrated at 50°C *in vacuo*

Experimental Animals

Ninety matured male albino rats of about twelve weeks old weighing between 130-281 g were used for the experiment. They were kept in a well-ventilated conventional cage 28-31°C, photoperiod of darkness 12 hours and 12 hours of natural light. The animals were allowed to acclimatize for two weeks and were fed on standard diet and had free access to water. The experiment was carried out according to the standard laboratory conditions as approved by the animal's ethical committee of the University of Uyo. The animals were divided into eight groups of six animals per group [5,6].

Hematological Assay

The extracts and fraction were administered using the outline;

Group I and II were given 10 mL and 1 mg/kg of distilled water and testosterone respectively.

Group III-V were administered crude stem bark extract at 44.72, 89.44 and 134.16 mg/kg respectively.

Group VI-VIII were given 89.44 mg/kg of n-hexane (n-Hexb), dichloromethane (DCMb) and 70% methanol (MTb) fractions daily for 7 days respectively.

The seed extracts were administered using the following outline;

Group IX-XI were given 86.60, 173.21 and 259.81 mg/kg/day of the crude seed extract respectively.

Group XII-XV received 173.21 mg/kg/day of n-Hexane (n-Hexs), Dichloromethane (DCMs), Methanol oil (MTOs) and 70% methanol (MTs) fractions for 7 days respectively. On the 8th day, the animals were anaesthetized with chloroform and sacrificed. The blood sample for hematological analysis were collected, via cardiac vein puncture stored in EDTA sample bottles and full blood count was determined using Mindray BC-5380 hematological analyzer [6-8].

Statistical Analysis

The results obtained were expressed as multiple comparisons of Mean±SEM. Significance was determined using one-way ANOVA followed by Tukey Kramer multiple comparison posttest with a p<0.05 was considered significant [5,13,14].

RESULTS AND DISCUSSION

Hematological effect of *Carapa procera* seed extract

The seed of *Carapa procera* extract showed insignificant effect on the white blood cell, neutrophil and basophil. However, on the lymphocyte the LDcs, MDcs, HDcs, MTOs, and MTs significantly suppresses the lymphocytes at p<0.01-0.001 as shown in Table 1 when compared to control. These suggest that the extracts could be used in the management of lymphocytic leukemia. This effect could also reduce the body immune system which could be due to its anti-inflammatory and immune suppressive potentials and could lead to lymphocytopenia and may predispose patient to recurrent viral, fungal and parasitic infection [2-11].

Monocytes are a type of leukocytes or white blood cells. They are the largest type of leukocyte and play essential role in vertebrate innate immune system. Also, monocyte influences the process of adaptive immunity. The n-hexane, dichloromethane and 70% methanol fractions significantly reduced blood concentration monocytes which could be used in conditions such as monocities a state of excess monocyte in peripheral blood which might be due to chronic inflammation, diabetes, viral fever, necrosis, severe infection, chronic myelomonocytic leukemia and advanced tuberculosis, malaria and lymphomas [9]. This showed that seed extract could have anti-inflammatory effect. The HDcs affects the eosinophils by significantly potentiating the blood level at p<0.05 when compared to control. The high blood concentration signified that the HDcs could cause allergic reaction [9-12].

The effect of the seed extracts (Table 3) on RBC, HGB, MCV, MCH and MCHC were insignificant compared to control. However, hemoglobin level, HDcs suppresses the production of hemoglobin which is an indication of anemia at p<0.05 for HGB and HCT when compared to control. This effect is not significant at low and median dose (LDcs and MDcs). RDW-CV and RDW-SD measures the range of variation of red blood cells and platelet (Table 5) showed insignificant effect [1-3].

Table 1: Effect of Seed extracts of *Carapa procera* on white blood cells

Sample	WBC (10 ⁹ /L)	Neut	Lymp	Mono	Eos	Baso (%)
VEH	12.04±1.42	32.63±3.29	62.50±3.17	4.18±0.84	0.82±0.14	0.87±0.11
STD	10.58±1.41	82.30±46.52	59.62±6.57	3.40±0.65	0.85±0.18	0.73±0.07
LDcs	14.46±1.24	61.28±2.60	34.68±2.47 ^b	2.35±0.17	0.90±0.10	0.78±0.11
MDcs	12.72±1.87	60.17±3.45	34.48±3.15 ^b	3.12±0.30	1.52±0.15	0.72±0.14
HDcs	12.56±2.13	61.65±3.14	33.15±2.82 ^c	2.92±0.27	1.55±0.22 ^a	0.73±0.11
n-Hexs	13.83±1.15	47.50±5.79	49.47±6.01	1.47±0.15 ^b	0.83±0.12	0.73±0.08
DCMs	13.64±1.28	43.85±6.14	52.93±5.99	1.43±0.22 ^c	0.90±0.12	0.88±0.08
MTOs	16.04±1.41	59.48±3.41	36.02±3.07 ^b	2.67±0.35	1.07±0.19	0.77±0.08
MTs	16.34±1.08	64.92±2.65	31.17±2.55 ^c	2.25±0.24 ^a	0.80±0.15	0.80±0.09

Values represent Mean±SEM, Significance relative to control; ^ap<0.05, ^bp<0.01, ^cp<0.001(n = 6)

Keys; WBC = White Blood Cell, Neut = Neutrophil, Lymp = Lymphocyte, Mono = Monocyte, Eos = Eosinophil, Baso = Basophil, VEH = Distilled water, STD = Standard drug (Testosterone), LDcs = Low Dose crude seed extract, MDcs = Median Dose crude seed extract, High Dose crude seed extract, n-Hexs = n-Hexane seed fraction, DCMs = Dichloromethane seed fraction, MTOs = Methanol seed oil, MTs = 70% Methanol fraction.

Table 2: Effect of stem bark extract of *Carapa procera* on white blood cells

Samples	WBC (x 10 ⁹ /L)	Neut	Lymph	Mono	Eos	Baso (%)
VEH	12.0±1.42	32.63±3.29	62.50±3.17	3.18±0.55	0.817±0.13	0.867±0.11
STD	12.04±1.41	35.35±6.02	59.62±6.57	3.40±0.65	0.850±0.18	0.733±0.07
LDcb	14.50±1.79	41.40±4.98	52.93±4.84	4.13±0.48	0.717±0.17	0.817±0.09
MDcb	11.23±0.79	36.36±4.98	59.02±2.29	3.02±0.29	0.767±0.17	0.833±0.09
HDcb	12.54±1.05	35.12±2.63	60.85±2.84	2.51±0.23	0.683±0.16	0.833±0.09
n-Hexb	17.09±3.28	25.32±3.78	69.50±3.45	3.72±0.33	0.617±0.19	0.850±0.13
DCMb	13.49±1.03	39.65±3.15	56.03±3.24	2.62±0.31	0.967±0.09	0.733±0.13
MTb	10.37±1.90	32.75±4.37	62.15±4.37	2.52±0.33	1.833±0.68	0.750±0.13

Values represent Mean±SEM, Significance relative to control; ^ap<0.05, ^bp<0.01, ^cp<0.001(n = 6)
 Key: VEH = Distilled water, STD = Standard drug (Testosterone); LDcb = Low Dose crude bark extract, MDcb = Median Dose crude bark extract, HDcb = High Dose crude bark extract, n-Hexb = n-Hexane stem bark fraction, DCMb = Dichloromethane stem bark fraction, MTb = 70% Methanol stem bark fraction

Table 3: Effect of the seed extracts of *Carapa procera* on red blood cells

Sample	RBC (x10 ¹² /L)	HGB (g/dl)	HCT (%)	MCV(FL)	MCH (pg)	MCHC(g/dl)	RDW-CV (%)	RDW-SD (FL)
VEH	7.68±0.10	13.95±0.18	44.88±0.59	58.47±0.47	18.17±0.15	31.08±0.11	14.27±0.24	34.68±0.62
STD	7.52±0.21	13.95±0.23	45.67±0.79	60.88±0.88	18.57±0.28	30.50±0.09	14.43±0.21	36.47±0.57
LDcs	7.23±0.15	13.15±0.27	44.65±1.00	61.72±1.02	18.17±0.20	29.43±0.26	15.21±0.41	39.02±1.48
MDcs	7.42±0.66	13.18±0.24	43.86±0.88	59.12±0.91	17.75±0.13	30.05±0.32	15.05±0.39	36.88±0.80
HDcs	6.68±0.66	11.63±1.07 ^a	37.53±3.48 ^a	56.80±0.89	17.50±0.20	31.05±0.19	14.15±0.28	33.18±0.88
n-Hexs	7.31±0.37	13.43±0.59	43.73±1.87	59.80±0.99	18.35±0.18	30.72±0.40	16.45±0.38 ^b	40.73±0.97 ^a
DCMs	8.09±0.16	13.95±0.23	45.63±1.01	56.48±0.83	17.27±0.18	30.58±0.26	16.38±0.44 ^b	38.25±1.62
MTOs	6.81±0.11	11.95±0.19	39.05±0.70	57.50±1.37	17.60±0.29	30.65±0.34	16.17±0.50 ^a	38.52±1.56
MTs	7.38±0.08	13.13±0.39	43.18±1.50	58.48±1.48	17.80±0.37	30.43±0.26	15.82±0.42	38.22±0.91

Values represent Mean±SEM, Significance relative to control; ^ap<0.05, ^bp<0.01(n = 6)
 Keys: RBC =Red Blood Count, HGB = Hemoglobin; HCT = Hematocrit, MCV =Mean Corpuscular Volume, MCHC = Mean Corpuscular Hemoglobin Concentration, RDW-CV = Red Cell Distribution Width coefficient of Variation, RDW-SD = Red Cell Distribution Width - Standard Deviation.

Table 4: Effect of stem bark extract of *Carapa procera* on red blood cells

Sample	RBC (x10 ¹² /L)	HGB(g/dL)	HCT (%)	MCV(FL)	MCH (pg)	MCHC (g/dL)	RDW-CV (%)	RDW-SD (FL)
VEH	7.68±0.10	13.95±0.18	44.88±0.59	58.47±0.47	18.58±0.28	31.08±0.11	14.27±0.24	34.68±0.62
STD	7.52±0.21	13.93±0.23	45.70±0.78	60.88±0.89	18.57±0.29	30.50±0.09	14.43±0.21	36.46±0.57 ^c
LDcb	8.03±0.18	14.75±0.25	50.38±1.16 ^a	62.82±0.93	18.37±0.16	29.28±0.37 ^c	15.91±0.30	41.37±1.01 ^c
MDcb	7.83±0.22	13.90±0.23	49.98±0.91 ^a	64.08±1.99 ^a	18.83±0.43	27.87±0.20 ^c	15.63±0.46	41.0±0.99 ^c
HDcb	8.22±0.24	15.03±0.27	52.33±1.38 ^c	64.08±1.989 ^a	18.33±0.32	28.75±0.32 ^c	15.62±0.24	34.70±0.63
n-Hexb	7.58±0.29	13.70±0.44	44.53±1.46	58.87±0.85	18.15±0.27	30.78±0.12	14.20±0.35	34.70±0.63
DCMb	7.19±0.29	12.88±0.27	42.78±0.88	59.93±1.47	18.00±0.52	30.0±0.21	15.57±0.69	36.53±1.47
MTb	7.69±0.29	13.77±0.35	45.82±1.39	59.65±0.78	17.93±0.23	30.05±0.36	14.17±0.24	35.05±0.72

Values represent Mean±SEM, Significance relative to control; ^ap<0.05, ^cp<0.001(n = 6)

Table 5: Effect of *Carapa procera* seed extract on blood platelet

Sample	PLT (x10 ⁹ /L)	MPV (FL)	PDW	PCT (ml/L)
VEH	870.83±61.0	6.42±0.13	15.02±0.06	5.59±0.40
STD	763.83±59.23	6.12±0.12	15.03±0.06	4.66±0.33
LDcs	728.67±28.79	6.00±0.07	15.28±0.04	4.37±0.16
MDcs	996.17±55.29	5.95±0.08	15.18±0.05	5.95±0.36
HDcs	694.33±145.5	6.23±0.19	15.08±0.09	4.22±0.83
n-Hexs	847.33±61.47	6.60±0.26	15.27±0.11	5.53±0.26
DCMs	884.67±25.31	6.30±0.11	15.12±0.05	5.50±0.19
MTOs	989.33±94.95	6.35±0.06	15.17±0.06	6.28±0.56
MTs	919.50±36.80	6.50±0.12	15.17±0.06	5.98±0.19

Values represent Mean±SEM, Significance relative to control; ^ap<0.05, ^cp<0.001(n = 6).

Keys; PLT Platelet, MPV = Mean platelet volume, PDW: Platelet Distribution width, PCT = Procalcitonin Test.

Table 6: The effect of Stem bark extract on platelet

Sample	PLT (x10 ⁹ /L)	MPV (fL)	PDW	PCT (m/L)
VEH	870.82±61.00	6.417±0.13	15.02±0.05	5.590±0.41
STD	763.83±59.23	6.117±0.19	15.03±0.06	4.658±0.33
LDcb	849.17±17.07	6.617±0.12	15.28±0.03 ^a	5.608±0.11
MDcb	875.33±50.29	8.483±0.25 ^c	15.47±0.07 ^c	7.430±0.07 ^a
HDcb	899.50±70.40	7.950±0.19 ^c	15.38±0.04 ^b	7.163±0.56
n-Hexb	759.83±101.0	6.117±0.09	15.23±0.05	4.630±0.59
DCMb	788.83±38.97	5.71s7±0.10 ^a	15.08±0.07	4.510±0.25
MTb	802.67±38.70	5.917±0.08	15.20±0.07	4.747±0.19

Values represent Mean±SEM, Significance relative to control; ^ap<0.05, ^bp<0.01, ^cp<0.001 (n = 6)

The effect of the seed extracts (Table 3) on RBC, HGB, MCV, MCH and MCHC were insignificant compared to control. However, hemoglobin level, HDcs suppresses the production of hemoglobin which is an indication of anemia at p<0.05 for HGB and HCT when compared to control. This effect is not significant at low and median dose (LDcs and MDcs). RDW-CV and RDW-SD measures the range of variation of red blood cells and platelet (Table 5) showed insignificant effect^[1-3].

Hematological effect of *Carapa procera* stem bark extracts

The stem bark extracts of *Carapa procera* showed insignificant effect on the leucocytes (Table 2), when compared to control (distilled water) while the effect on erythrocytes; RBC, HGB are not significant as shown in Table 4. However, LDcb, MDcb and HDcb increased the level of hematocrit which could be as a result of dehydration, disorder bone marrow, polycythemia rubra, lung disease at p<0.05 and 0.001 when compared to control^[9]. The MDcb and HDcb significantly increased MCV at p<0.05 which showed that the animals are anemic, while LDcb, MDcb and HDcb significantly reduced MCHC at p<0.001 when compared to control. This implies that the red blood cells hemoglobin concentration is low. The deficiency of iron in the blood may indicate a sign of anemia, which could lead to fatigue and inflammatory disorder. The LDcb and MDcb extracts significantly increased RDW-SD at p<0.001 when compared to control however the extracts showed insignificant effect on RDW-SV. The crude extracts and fraction showed insignificant effect on the white blood cells when compared to the negative control, and insignificant effect on platelet (Table 6) ^[1-12].

CONCLUSION

The seed extract showed significant suppressive effect on the lymphocytes which could be used in lymphocytic leukemia and may suppress immune system in healthy individual who is using this medicinal plant and predispose to infectious diseases while the stem bark showed insignificant effect on the lymphocyte rather it increased the level of hematocrit which could be due to dehydration and predispose the rodents to anemia.

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Conflict of Interest

None declared.

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