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Research Article

LEFT VENTRICULAR CARDIAC BLOOD ANALYSIS IN MALE WISTER RATS TREATED WITH AQUEOUS EXTRACT OF *CARICA PAPAYA* BARK

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ABSTRACT

Introduction: We have examined the virtues of Carica papaya (paw-paw) bark extract as an antifertilty agent in male rats. The corresponding consequence of the extract on blood factors in laboratory animals is yet to be studied.

Aim: To investigate the effect of aqueous extract of Carica papaya (CP) bark on hematological parameters in male Wister rats

Methodology: Sixty male Wister rats weighing between 115-150 g were assigned to three Groups I, II & III consisting of 10, 20 and 30 rats respectively. Group I & II rats were fed aqueous CP extract at 50 & 100 mg ml^{-1} day^{-1} for 4 & 8 weeks. Group III (control) treated with distilled water. At the end of 4 weeks rats in Group I were sacrificed. In Group II, 10 rats were sacrificed at the end of 8 weeks; the other 10 at the end of 16 weeks after withdrawal of extract for 8 weeks. In Group III, rats were used to contrast events in the experimental groups. Complete blood count (Red blood cell and parameters; White blood cell and differential and Platelet count) were estimated for all the blood samples collected from sacrificed rats.

Results: The extract did not significantly ($p > 0.05$) affect the levels of Red blood cell and platelet counts. Conversely, low dose aqueous extract of CP (50 mg/kg/ml) resulted in a significant decrease ($p < 0.05$) in total leukocytes count.

Conclusion: This study has shown that administration of CP bark extract appears to be relatively non-toxic to animals if considered for use as a male contraceptive agent.

Keywords: *Carica papaya*, Red blood cell, White blood cell, Platelet count, Wister rats

INTRODUCTION

Some plant-derived compounds have been found to affect fertility. The exploit of plant products to regulate fertility is of a primeval origin. In spite of numerous studies, no plant with confirmed contraceptive efficiency but devoid of toxicity has emerged so far[1]. A promising oral compound would allow metabolism by the liver and allow reduction of dose below toxic level. Examples of plants (herbs) reported to possess anti-fertility properties are date palm, oil palm, wheat and *Carica papaya* (CP) [2]. CP is different because it has been documented to improve a wide range of variety of health conditions[3]. CP has been widely used throughout history for its medical value to human for different conditions except as male contraceptive[4]. Different authors have reported the use of its fruit, seed, leaf and root as a principle in a number of biological conditions[5, 6]. However, there is a dearth in the literature concerning the bark of the plant. In our previous work we established the use of aqueous extract of CP bark as an effective oral male contraceptive agent[7] in experimental rats. In the study, the rats were fed the aqueous extract of CP bark at 50 and 100 mg ml^{-1} day^{-1} and 4-8 weeks duration[7]. The present study attempts at taking the investigation a step further to include the effect of aqueous extract of CP on the hematological parameters on these male rats and a possible reversal if any. So far, little is known about the hematological effect of the bark extract of CP. The plant is tropical American in origin, but now widely spread throughout tropical Africa[8]. It is a small perennial herbaceous plant that could reach a height of 10 meters. CP of the *Caricaeae* family belongs to a group of plants called lacticiferous plants[9].

MATERIALS AND METHODS

Experimental animals

Sixty mature male Wister rats (6-8 weeks old) weighing between 115-150 g were used for the study. They were bred and reared in the animal holding unit of Anatomy department, College of Medicine, University of Lagos, Nigeria. They were authenticated by a taxonomist[10] at the Zoology Department of the same University. Rats were housed in well-ventilated metal cages at temperatures of 29–30^{o}C and a relative humidity of 50–55%. They were exposed to a photo period of 12 h light, alternating with 12 h darkness; rat chow (Livestock feeds Plc. Ikeja, Lagos, Nigeria), and clean tap water were provided *ad libitum*.

Carica papaya bark extracts preparation

The bark of the *Carica papaya* plant was obtained from a forest in Lagos and authenticated by a taxonomist in the Department of Botany, University of Lagos. A voucher specimen (code: LUH 2151) was deposited in the institutions herbarium. The bark of the plant was dried in an electric oven at 40°C for 4 days. The extract preparation was done according to methods described by Olatunji 2005[11]with a slight modification. Briefly; 50 g of the bark material was crushed to obtain a coarse powder that was used for the extraction in the Pharmacognosy Department of the Faculty of Pharmacy, University of Lagos. It was kept in 500 ml of distilled water for 24 h at the same temperature. The filtrate was thereafter obtained from the solution using the whatman no 1 filter paper and evaporated to dryness in an oven at 60^{0}C. The residue of the extract obtained in the form of paste was stored in a capped bottle and kept in a desiccator[11].

Research protocol and autopsy schedule

The animals were randomly allocated into three Groups I, II and III comprising 10, 20 and 30 rats respectively. Group I rats were fed with low dose of aqueous CP extract (50 mg ml^{-1} day^{-1}) for 4 weeks. Group II rats treated with high dose of aqueous CP extract (100 mg ml^{-1} day^{-1}) for 8 weeks. Group III served as controls were fed equal volumes (2-5 ml) of distilled water. Administration of the extract/distilled water was by orogastric gavages using a metal canula. At the end of 4 weeks duration all the rats in Group I were sacrificed. In Group II, half of the rats were sacrificed at the end of 8 weeks interval; the remaining half were fed with distilled water for another 8 weeks. They were examined for reversibility effect and sacrificed at the end of 16 weeks. In Group III, 10 rats were sacrificed at the end of 4, 8 and 16 weeks respectively were used to compare events in I and II. In all, the rats were sacrificed by cervical dislocation under chloroform anesthesia.

Blood collection

Intra-necropsy blood samples were obtained by left ventricular cardiac puncture for the haematological studies. Blood was collected

immediately into EDTA specimen bottles. All samples were stored in a refrigerator at 4^0C until the time of analysis. The complete blood count (CBC) estimated for all samples were;

a) Red blood cell (RBC), packed cell volume (PCV) or hematocrit, hemoglobin (HGB), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC)
b) Total white cell count (WBC), percentage lymphocytes (LYM%), mixed cell i.e. eosinophil, basophil & monocyte, percentage (MXD%), neutrophil percentage (NEUT%), lymphocyte number (LYM#), mixed cell number (MXD#), neutrophil number (NEUT#)
c) Platelet count (PLT) respectively. The haematological parameters were evaluated by computerized hematological analyzer (SYSMEX KX–21, Supplied by SYSMEX Corporation, Japan) [11] using whole blood samples.

Statistical Analysis

The data are expressed as Mean ± SD. Significant was determined using the student's t-test. *P*-values less than 0.05 were considered statistically significant. All animal experiments were conducted incompliance with NIH guidelines for Care and Use of Laboratory Animals[12].

RESULTS AND DISCUSSION

The present study was modified to underscore the effect of the extract on the blood parameter in experimental rats treated with graded doses of CP bark extract that produced contraceptive effects in male rats[7]. The results obtained depicted a selective toxicity on some hematological parameters. A complete blood count, also known as full blood count (FBC) or full blood exam (FBE) or blood panel, is a test panel that gives information about the cells in the experimental animal. The cells circulating in the bloodstream are generally divided into white blood cells (leukocytes), red blood cells (erythrocytes), and platelets (thrombocytes).

The effect of oral administration of the aqueous bark extract of CP at the doses investigated on erythrocytes and its functional indices in male Wistar rats for 4 and 8 weeks as well as reversibility are shown in (Table 1). The extract did not significantly alter the levels of RBC, PCV, HGB, MCV, MCH and MCHC. The absence of changes on these indices suggests that the extract does not possess toxic substances that can cause an anemic condition in rats. It also means the extract may not have had any adverse effect on the bone marrow, kidney and haemoglobin metabolism, since the value of red blood cells are not greatly affected[13]. Similarly, the increase in MCV (although not significant), as shown in this study, also agrees with the incapability of the extract to induce anemic conditions in animals[14].

Table 1: Effect of graded doses of *Carica papaya* bark extract (mg ml^{-1}day^{-1}) on erythrocyte and platelet indices in experimental and control male Sprague-Dawley rats

Duration	Group	Dose	RBC	HGB	PCV	MCV	MCH	MCHC	PLT
4 weeks	I	50	4.5±1.3	8.2±2.8	26.3±8.8	57.9±2.4	18.0±0.8	31.1±0.1	410.5±379.7
	II	100	5.9±0.3	10.6±0.1	35.1±0.1	58.5±2.5	17.9±0.6	30.8±0.2	271.7±304.6
	III	2-5 ml	7.0±0.9	11.5±2.1	37.2±2.7	49.8±2.1	16.8±0.1	35.0±2.5	429.4±4.7
8 weeks	I	50	7.5±0.2	12.8±0.3	43.7±1.2	58.3±2.9	17.1±0.8	29.3±0.2	531.6±134.9
	II	100	8.1±0.1	13.7±0.5	47.0±3.0	58.4±4.8	17.0±0.9	29.1±0.8	575.5±50.2
	III	2-5 ml	7.2±0.2	13.2±0.4	42.2±2.7	65.2±7.2	17.6±1.0	33.7±4.3	417.4±21.7
16 weeks	I	50	6.0±0.8	11.1±1.3	35.8±4.7	59.3±0.5	18.3±0.4	30.9±0.4	575.0±147.1
	II	100	7.1±1.0	10.9±1.2	47.8±6.6	62.0±3.4	20.2±2.2	32.5±1.9	510.0±239.0
	III	2-5 ml	7.9±0.5	13.2±0.4	42.2±1.3	65.8±5.2	16.8±1.6	33.3±9.5	417.4±21.3

*$p < 0.05$ compared with Group III (control); RBC = Total red blood cell count (x 10^6/ml), HGB = Hemoglobin concentration (dl); PCV = Packed cell volume (%); MCV= Mean cell volume (Fl); MCH: Mean cell hemoglobin (pg); MCHC: Mean cell hemoglobin concentration (al); PLT= Platelet count (10^3/ml)

Table 2: Effect of graded doses of *Carica papaya* bark extract (mg ml^{-1}day^{-1}) on white cell count and differential in experimental and control male Sprague-Dawley rats

Duration	Group	Dose	WBC	LYM%	MXD%	NEUT%	LYM #	MXD #	NEUT#
4 weeks	I	50	10.7±6.5	36.9±0.7*	13.5±2.2	53.3±3.7*	3.9±0.4*	1.3±0.2	8.9±1.6*
	II	100	6.2±1.9	47.7±3.0*	5.9±1.2	46.0±1.4*	2.9±0.7*	0.3±0.2*	2.9±1.0
	III	2-5 ml	9.0±3.7	71.3±2.3	8.9±0.4	21.9±4.7	6.7±0.3	1.0±0.0	2.4±0.4
8 weeks	I	50	7.6±1.8*	66.5±0.4*	9.4±1.6	24.1±2.0*	5.1±1.2	1.1±0.3	2.3±0.4
	II	100	14.3±0.3	65.7±7.0	7.0±4.9	27.4±11.9	9.4±1.1	1.1±0.8	3.9±1.6
	III	2-5 ml	16.2±1.6	41.4±4.1	4.8±0.9	52.8±4.7	7.5±1.1	1.3±0.5	5.7±2.4
16 weeks	I	50	6.6±0.0	38.6±8.1	7.7±2.0	52.7±4.6	2.5±0.7*	0.7±0.4	4.0±1.0
	II	100	9.1±0.6	39.8±7.1	6.7±0.6	49.7±1.0	3.4±0.6*	2.2±1.9	3.9±0.4
	III	2-5 ml	17.7±3.7	42.4±2.7	5.8±0.5	54.8±7.6	7.5±1.2	1.3±0.5	6.7±1.0

*$p < 0.05$ compared with Group III (control); WBC= Total white cell count; LYM%= percentage lymphocytes; MXD%= mixed cell (eosinophil, basophil & monocyte), percentage; NEUT%= neutrophil percentage; LYM#= lymphocyte number; MXD#= mixed cell number; NEUT#=neutrophil number

The low dose aqueous extract of CP (50 mg/kg/ml) resulted in a significant decrease ($p < 0.05$) in total leukocyte count from 16.2±1.6 to 7.6±1.8 x 10^6 by 8 weeks. These results gainsay the findings of Swenson and Reece[15], who reported that toxic plants do not produce a direct effect on leukocytes and its functional indices. However, the result obtained from this study shows otherwise. The leukocyte differential (Table 2); the number & percentage lymphocytes fed low and high doses of the extract for 4 weeks were significantly decreased ($p < 0.05$) compared to control. The values were 3.9±0.4 & 36.9±0.7 *vs.* 2.9±0.7 & 47.7±3.0 compared to controls 6.7±0.3 & 71.3±2.3 fed distilled water. These values became elevated after 8 weeks of extract treatment for both the low (5.1±1.2 & 66.5±0.4) and high (9.4±1.1 & 65.7±7.0) doses compared to control (7.5±1.1 & 41.4±4.1). However the value was only significant ($p < 0.05$) for the low dose. The lymphocyte number did not recover (values remained decreased) after 8 weeks of extract discontinuation. The neutrophil

number & percentage were elevated from 2.4±0.4 & 21.9±4.7 (control) to 8.9±1.6 & 53.3±3.7 (low dose) 2.9±1.0 & 46.0±1.4 (high dose) in rats fed CP extract for 4 weeks. For the 8 weeks duration, values were depressed from 5.7±2.4 & 52.8±4.7 to 2.3±0.4 & 24.1±2.0 (low dose) & 3.9±1.6 & 27.4±11.9 (high dose). This percentage increase of neutrophil may be adduced to the ability of the animals to ingest offending agents. A similar effect has been reported on the use of drugs such as histamine and epinephrine[16]. The neutrophil counts were higher and lymphocyte counts were lower in the extract treated groups, an indication of an immunosuppressive effect. At withdrawal of the extract, values became comparable to baseline control for both concentrations.

The mixed cell number (eosinophil, basophil & monocyte) followed a slightly different pattern compared to control baseline of 1.0±0.0. Mean values were not affected in rats fed low doses of CP extract but depressed significantly to 0.3±0.2 ($p < 0.05$) in those fed at higher dose for 4 weeks. The decreased mixed cell number might have resulted from the suppression of leukocytes in the bone marrow and thus may have consequential effects on the immune system and phagocytic activity of the animals[17]. However at longer durations for both doses mixed cell number were not affected. At reversibility, values mirrored their control counterpart.

Platelets play a crucial role in reducing blood loss and repairing vascular injury[18]. There were no significant alterations observed in the platelet count. This implies that the extract had no effect on the biosynthesis/function of clotting factors.

In conclusion, this study has shown that administration of CP bark extract appears to be relatively non-toxic to animals if considered for use as a contraceptive. This is because there was no apparent damage to the hematological indicators that assess organ-specific toxicity except the leukocytes and its relative indices. Meanwhile, the alterations observed on some parameters suggest parameter and dose selective toxicity of the plant when repeatedly consumed on a daily basis at the doses investigated for 4-8 weeks. However, further work is needed for the identification of the active components to provide mechanisms of action of the active constituents of the extract.

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