Effect of *Carica papaya* (L) Leaves on Haematological Parameters in Ccl₄-induced Wistar Albino Rats

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Authors’ contributions

This work was carried out in collaboration between all authors. Author OJS designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author ARA managed the literature searches, analyses of the study and author KK managed and interpret the haematolgical experimental process. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2016/13686

Editor(s):
(1) Chan-Min Liu, School of Life Science, Xuzhou Normal University, Xuzhou City, China.
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Complete Peer review History: http://sciencedomain.org/review-history/14908

Received 28th August 2014
Accepted 24th May 2016
Published 4th June 2016

ABSTRACT

Aims: To investigate the effect of *Carica papaya* leaves on some haematological parameters (PCV, RBC, Hb, WBC and differential blood counts) were investigated.

Place and Duration of Study: Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa-State, Nigeria, between.

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Methods: Thirty male albino rats were randomly allotted to five groups of six rats per group. Haemoglobin (Hb) was determined spectrophotometrically by the cyanomet haemoglobin method. Red blood cells (RBC), was estimated by haemocytometer, using adopted standard procedure. Group 1 (negative control) were fed with 100% rat feed. Groups 2-4 were pretreated with 10, 30 and 50% Carica papaya L respectively, while Group 5 (normal control) received 100% rat feed. Rats in groups 1-4, were injected with CCl₄ (0.5 ml/kg body weight in 0.5 ml olive oil) on the 29th day while rats in group 5 were not administered with CCl₄ (normal control).

Results: The results were statistically analyzed using one-way analysis of variance (ANOVA). There were significant increases (p ≤ 0.05) in the levels of Hb, PCV, RBC, lymphocytes and decreased WBC and neutrophils in rats in group 5 (normal control) as against the negative control (group 1). Rats groups pretreated with 10, 30 and 50% Carica papaya (groups 2, 3 and 4 respectively), showed significant increases (p ≤ 0.05) PCV, RBC and Hb levels, when compared with untreated rats (group 1). Rats that were administered with CCl₄ only (negative control), showed significant increases (p ≤ 0.05) in the levels of WBC and neutrophils. However, incorporation of 10, 30 and 50% Carica papaya in groups 2, 3 and 4 respectively, significantly decreased the levels of WBC and neutrophils, when compared with rats in untreated group 1. Monocytes levels significantly increased (p ≤ 0.05) in rats pre-treated with 30% and 50% Carica papaya (groups 3 and 4 respectively). While, there was zero level of basophils in all the groups.

Conclusion: Carica papaya L, may therefore possess and confer erythropoietic properties on rats pretreated groups as evident in the increased levels of Hb, PCV, RBC and lymphocytes.

Keywords: Carica papaya L; hemoglobin; lymphocytes; white blood cell counts; wistar albino rats.

1. INTRODUCTION

Carica papaya Linn is the most widely cultivated and best known species. The papaya tree belongs to a small family—Caricaceae having four genera in the world. Other known species include; C. cauliflora Jacq; C. pubescens Lenne and K. Koch and C. quercifolia Benth. f. exttieron. It is a tree reaching 3-10 m in height, with the habit of a palm; the fleshy stem marked by scars where leaves have fallen off, is surmounted by a terminal panache of leaves on long petioles and with 5-7 lobes [1]. Flowers fragments are trimorphous, usually unisexual dioecious, male flowers in lax many-flowered and densely pubescent cymes at the tips of the pendulous, fistular rachis. Female flowers are large, solitary or in few flowered racemes, with a short thick rachis. The fruit is a large berry, varying widely in size, elongate to globose with a large central cavity, seeds are black, tuberculous and enclosed in a transparent aril. The fruit bearing trees are less than 18 month old. Papaya is a man’s common fruit and has a high nutritive value. It is low in calories and rich in natural vitamins and minerals. Papaya ranks among the first fruits for vitamin C, vitamin A, riboflavin, foliate, calcium, thiamine, iron, niacin, potassium and fibre [1]. The fermented papaya fruit is a promising nutraceutical as an antioxidant. It was reported to improve the antioxidant defense in elderly [2]. The fruit is rich in vitamins, minerals, proteins, polysaccharides, lectins, saponins and flavonoids, and can be used in the prevention of complications of diabetes mellitus [3]. Biological activities of papaya are reported with the crude extracts and different fractions from latex, seed, leaf, root, stem, bark and fruit. However, crude extracts of different parts of papaya have been used as traditional medicine for the treatment of various diseases [4,5]. Young leaves have been reported by various authors to possess variety of uses such as, vegetable, jaundice (fine paste), treatment of urinary complaints and Gonorrhea (infusion), dressing wounds (fresh leaves), antibacterial activity, antimicrobial activity, abortion (infusion), asthma (smoke) [6-10]. Inspite of these numerous uses, little has been reported on the effect of the plant on its haematological benefits. Hence, the need to investigate the effect of Carica papaya leaves on some haematological parameters on carbon tetrachloride induced wistar albino rats.

2. MATERIALS AND METHODS

Fresh samples of C. papaya leaves were collected from the herbal garden of Department of Pharmacognosy, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The plant was identified and authenticated by a Pharmacognosist, Prof S. K. Adesina of Department of Pharmacognosy and was deposited in the herbarium with voucher no: NDUP 093. The leaves were detached from the stems, washed twice with distilled water to
remove adulterants, dried under natural conditions for two weeks, ground into powder using an electric blender and stored in airtight containers.

2.1 Animals

Thirty (30) male wistar albino rats weighing (180–190) g were obtained from the animal house unit of the Department of Pharmacology, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The animals were distributed into stainless metabolic cages (five groups with six rats per cage) and observed under a 12-hour/12-hour light/dark cycle in a well ventilate room at 26–27°C. They were fed with standard rat chow (Bendel Feed and Flour Mill Limited, Ewu, Benin City, Nigeria) and water ad libitum. The acclimatization period lasted for 7 days. Permission and approval for animal studies were obtained from College of Health Sciences, Animal Ethics Committee, Niger Delta University with Ref: No. NDU/CHS/FBMS/0078.

2.2 Phytochemical Screening

In 2008, Krishna et al. [1] reported the chemical composition of *C. papaya* to include; alkaloid, α-carbaine, β-D-glucosides, β-sitosterol, papain, choline, carotene, riboflavin, vitamin C, phenyethyl-β-D-glucosides, amongst others.

2.3 Experimental Design

Thirty (30) albino rats (180-190 g) were randomly divided into five groups comprising of six rats each. Animals were fed with substances under investigation for a period of twenty eight (28) days after which they were injected with 0.5 ml/kg of CCl4, dissolved in 0.5 ml of olive oil. They were fed *ad libitum* with free access to water. Group 1 (negative control) were fed with 100% rat feed. Groups 2- 4 were pretreated with 10, 30 and 50% *C. papaya* respectively, while Group 5 (normal control) received 100% rat feed. Rats in groups (1-4), were injected with CCl4 (0.5 ml/kg body weight in 0.5 ml olive oil) on the 29th day while rats in group 5 were not injected with CCl4 (normal control). The animals were fasted for 24 hrs before sacrified on the 30th day of the study.

2.4 Sample Collection

Twenty-four hours after the last administration, the animals were anaesthetized with chloroform vapour and dissected. Whole blood was obtained by cardiac puncture from each rat and collected into anticoagulant – treated (EDTA 0.77M) sterile bottles. This was used for haematological studies. Blood haemoglobin (Hb) was determined spectrophotometrically by the cyanomethae-moglobin method [11], packed cell volume (PCV) was determined by method of Jain [12]. Red Blood Cell Count (RBC), was estimated by haemocytometer method of [12,13]. Blood was diluted in 1,200 Dacie’s fluid which keeps and preserves the integrity of the RBC. White Blood Cell (WBC) counts [12], the dilution factor was 1:20 using 2-3% solution of acetic acid to which gentian violet was added. The calculations for red cell indices were made as described [11]:

Mean Corpuscular Volume
MCV (fl) = PCV (1/1) / RBC count (×10-12)

Mean Cell Haemoglobin
MCH (pg) = Hb (g/dl) / RBC count (×/10-12/1)

Mean Cell Haemoglobin Concentration
MCHC (g/dl) = Hb (g/dl)/ PCV(1/1)

2.5 Statistical Analysis

The data of liver and body weights and biochemical analysis were analyzed using the Statistical Package for Social Sciences (SPSS for window, version 12.0). Comparism was made between control and experimental groups using ANOVA test. Values of less than 0.05 were regarded as statistically significant.

3. RESULTS

The haematological parameters that were used to assess the effects of *Carica papaya* on the blood of rats include: PCV, Hb, RBC, MCH, MCV, MCHC, WBC, lymphocytes, neutrophils, eosinophils, monocytes and basophils, in CCl4-induced hepatic injury in wistar albino rats are shown in Tables 1 and 2. The results suggested that the CCl4 free groups 5 positively improved the blood components. This observation was reflected by significant increases (p≤0.05) in the levels of Hb, PCV, RBC, lymphocytes and decreased WBC and neutrophils. Treatment of rats with CCl4 significantly decreased (p≤0.05) the levels of PCV, RBC and Hb in negative control while groups pretreated with 10, 30 and 50% *Carica papaya* (groups 2, 3 and 4 respectively), significantly increased (p≤0.05) PCV, RBC and Hb levels, when compared with untreated rats (group 1) as shown in Table 1.
Table 1. The effects of *C. papaya* on some hematological parameters in CCl\(_4\) induced wistar albino rat

| Group | Treatments                  | Hb (g/dl)  | PCV (vol%) | RBC×10\(^6\) µl | MCH (pg)   | MCV (µm\(^3\)) | MCHC (%) |
|-------|-----------------------------|------------|------------|-----------------|------------|----------------|----------|
| 1     | 100%Feed +CCl\(_4\)         | 14.55±1.47 | 43.75±4.49 | 8.80±1.10       | 16.53±1.00 | 49.72±2.00     | 33.26±2.00 |
| 2     | 90%Feed+10% *C. papaya* + CCl\(_4\) | 15.30±3.36 | 46.00±9.54 | 9.50±1.01       | 16.11±1.00 | 48.42±2.00     | 33.26±2.00 |
| 3     | 70%Feed+30% *C. papaya* + CCl\(_4\) | 16.55±1.77 | 50.75±3.34 | 9.75±1.10       | 16.97±1.00 | 52.05±2.00     | 32.61±2.00 |
| 4     | 50%Feed+50% *C. papaya* + CCl\(_4\) | 17.63±1.77 | 58.25±6.60 | 9.90±1.00       | 17.81±1.00 | 58.84±2.00     | 32.61±2.00 |
| 5     | 100%Feed (normal control)    | 18.65±2.86 | 65.00±1.00 | 10.50±1.10      | 17.76±1.00 | 61.90±2.00     | 28.69±2.00 |

Values are means ± S.D (n=6), n equals to sample size.

Means with different superscripts are significantly different at the 0.05 levels in columns.

Table 2. The effects of *C. papaya* on some hematological parameters in CCl\(_4\) induced wistar albino rat

| Group | Treatments                  | WBC x 10\(^9\)/L | L (Lymphocytes) | N (Neutrophils) | E (Eosinophils) | M (Monocytes) | B (Basophils) |
|-------|-----------------------------|-------------------|----------------|----------------|----------------|---------------|---------------|
| 1     | 100%Feed +CCl\(_4\)         | 10.50±1.00        | 40.40±11.60    | 57.80±10.59    | 1.00±0.01      | 1.00±0.01    | -             |
| 2     | 90%Feed+10% *C. papaya* + CCl\(_4\) | 7.85±1.11        | 52.00±1.01     | 45.00±1.01     | 3.00±0.01     | -             | -             |
| 3     | 70%Feed+30% *C. papaya* + CCl\(_4\) | 7.24±1.00        | 54.60±2.40     | 43.80±1.50     | 0.50±0.01     | 2.00±0.01    | -             |
| 4     | 50%Feed+50% *C. papaya* + CCl\(_4\) | 6.95±1.24        | 54.75±5.61     | 42.75±0.01     | 0.50±0.01     | 2.00±0.01    | -             |
| 5     | 100%Feed (normal control)    | 6.05±1.00        | 64.00±2.40     | 36.00±1.10     | -              | -             | -             |

Values are means ± S.D for 6 replicates (n=6).

Means with different superscripts are significantly different at the 0.05 levels in columns.

L (Lymphocytes), N (Neutrophils), E (Eosinophils), M (Monocytes), B (Basophils)
Rats that were administered with CCl₄ only (negative control), showed significant increase (p ≤ 0.05) in the levels of WBC and neutrophils. However, incorporation of 10, 30 and 50% Carica papaya in groups 2, 3 and 4 respectively, significantly decreased the levels of WBC and neutrophils, when compared with rats in untreated group 1 (Table 2). The eosinophils levels showed significant increase (p ≤ 0.05) in rats that were pre-treated with 10% Carica papaya. Whereas, rats groups that were pre-treated with 30% and 50% Carica papaya showed significant decrease (p ≤ 0.05) when compared with rats that received CCl₄ only. Monocytes levels significantly increased (p ≤ 0.05) in rats pre-treated with 30% and 50% Carica papaya (groups 3 and 4 respectively). While, there was zero level of basophils in all the groups.

4. DISCUSSION

Hematological studies on some vital hematological parameters (Hb, PCV, RBC, MCH, MCV, MCHC, WBC and differential counts) were carried out during the chronic toxicity study in rats due to their roles in providing reliable information concerning hematological changes toxicants could cause. Hematological parameters have been associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health [14]. The plants Carica papaya L produced significant increases in PCV, Hb, RBC, MCV, MCH and lymphocytes. There were decreases in the levels of WBC, MCHC and neutrophils when compared with rats administered with CCl₄ only (group 1). This agrees with an earlier report observed by Fairbark in 1967 [15], that xenobiotics can cause haemolytic anaemia when sulphydryl groups of the erythrocyte membrane is oxidized which inflicts injury to the erythrocyte membrane. Carica papaya was reported to rank among the first fruits for vitamin C, vitamin A, riboflavin, foliate, calcium, thiamine, iron, niacin, potassium and fibre and may probably be responsible for Carica papaya conferring erythropoietic properties on the pretreated rats [1]. This haemorrhage caused by CCl₄ in liver was minimized by the use of plant (Carica papaya) as flavonoids, one of its active components are known to be vasculo protector and a powerful antioxidant [1]. Earlier report made by Oladunmoye and Osho in 2007 [16], revealed that the higher values of these haemological indices in rats pretreated with plants Carica papaya may be due to the inability of CCl₄ to cause haemolysis resulting from the anti-inflammatory potentials inherent in the herbs. The increased values of RBC and associated parameters (Hb, PCV) are also suggestive of polycythemia [17]. Therefore, as observed in this present study, the herbs of Carica papaya at (10, 30 and 50%) may not have had any adverse effect on the bone marrow and haemological metabolism, since the values of red blood cells are not negatively affected thus corroborating report ascerted by Young and Maciejewski in 1997 [18]. Findings from this study is in agreement with the earlier reports by Oladunmoye and Osho, [16] that Hb and PCV levels increased significantly in rats orogastrically dosed with Salmonella typhi and Staphylococcus aureus and treated with ethanolic leaf extract from Carica papaya. The values of lymphocytes increased in rats pretreated with Carica papaya when compared with rats administered with CCl₄ only. This may go a long way to suggesting that this plant may have influenced the defense mechanism of the test rats. So, the continuous exposure of the body systems of animals to these medicinal products (herbs) may cause lymphocytosis, which may then account for the use of these plants for medicinal purposes [19]. The increases in WBC and neutrophils in CCl₄ treated rats observed in this study (group1), Table 2, may be considered as a defensive mechanism by the immune system which corroborates the report by Hoeney, [20] that when an antigen is introduced into an organism, antibodies are produced in response to the antigen. This can also be related to the findings of Adisa et al. [21], who observed an increase in WBC and a corresponding decrease in PCV values in albino rats infected with T. brucei. Neutrophils are the most abundant circulating granulocytes and their granules contain numerous microbicidal molecules and when a chemotactic factor is produced as a result of infection or injury in an extracellular site, these cells enter the tissues [22].

5. CONCLUSION

In conclusion Carica papaya leaves, may therefore possess and confer erythropoietic properties on pretreated rat groups as evident in the increased levels of Hb, PCV, RBC and lymphocytes.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.
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