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Biochemical evaluation of chloramphenicol-induced lymphoma and ameliorative potentials of *Justicia carnea* and *Cnidocolus aconitifolius* in male Wistar rats

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Abstract

The biochemical implication of chloramphenicol-induced lymphoma and the ameliorative potential of *Justicia carnea* and *Cnidocolus aconitifolius* on male Wistar rats were evaluated in this study. Seventy (70) male Wistar rats with average body weights of 128g were randomly grouped into 14 of 5 rats each. Group 1 received commercial rat feed and water *ad libitum*. Rats in groups 2-14 were given 250mg/kg bodyweight of chloramphenicol by oral intubation for 28 days. Results of blood samples collected after 28 days from all the groups showed normal blood film in Group 1 and abnormal increases and lymphocytes and the presence of blast cells in Groups 2-14. Group 2 did not receive any treatment and is referred to as the negative control. The remaining groups (3-14) were administered with aqueous leaf extracts of *J. carnea* (Groups 3-6), *C. aconitifolius* (Groups 7-10), and a combination of both extracts (Groups 11-14) in doses of 500mg/kg, 1000mg/kg, 1500mg/kg and 2000mg/kg respectively, for 28 days. Data obtained showed that lymphocytes were elevated ($p < 0.05$) in Group 2 lymphocytosis and there was the presence of blast cells indicating lymphoma when compared to Group 1. The combination of *J. carnea* and *C. aconitifolius* was able to ameliorate the chloramphenicol-induced lymphoma better than the single therapy of each extract. The result of the investigation supports the earlier findings that chloramphenicol could cause acute lymphocytic leukemia. It further provides evidence that combined extracts of *J. carnea* and *C. aconitifolius* may have an ameliorative effect in blood diseases connected to over-exposure to chloramphenicol.

Keywords: *Justicia carnea*; *Cnidocolus aconitifolius*; Chloramphenicol; Lymphoma; Photomicrographs

1. Introduction

Blood is a bodily fluid that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells. There are three major categories, each of which has specific functions [1]. The white blood cell (WBCs), or leukocytes, engage in the defense of the body against foreign microorganisms or toxic substances as well as mediate the immune response. The WBCs are subdivided further into three groups; granulocytes, monocytes and macrophages, and lymphocytes (T-cells and B-cells). The platelets help maintain the integrity of the blood vessels by stimulating blood clotting (i.e., coagulation) after an injury. Red blood cells (RBCs), also called erythrocytes, transport oxygen from the lungs to all the cells in the body and carry carbon dioxide from the cells back to the lungs [1].

Lymphoma is a type of blood cancer that happens when something goes wrong with the development of a type of white blood cell called lymphocytes found in the blood, bone marrow, and lymph glands. Man has been using herbs and plant products in combating diseases since time immemorial. The traditional system of medicine has been such that larger percentages of the populations in Africa and Asia depend on the indigenous system for the relief of symptoms of various diseases. Regular consumption of plant foods is associated with numerous health benefits rooted in their various

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physiological effects as a result of their phytochemical and nutritional constituents [2]. Green leafy vegetables are particularly important in promoting health because of their rich content of nutrients [3]. A good number of medicinal plants are traditionally employed in alleviating blood diseases. Some of these plants include; *Telfeira occidentalis*, *Combretum dolichopetalum*, *Psorospermum ferbrifugum*, *Jatropha curcas*, *Flacourtia flavescens* and *Brillantasia nitens* [4, 5], and *Justicia carnea*. The leaves of *J. carnea* are commonly used as haematinic and claimed to be very effective. This has led to the biochemical investigation of the plant. *C. aconitifolius* which belongs to the family Euphorbiaceae is an evergreen, drought-deciduous shrub up to 6m in height with alternate palmate lobed leaves and milky sap. The leaves are large, 32cm x 30 wide on the succulent petiole. It originated as a domesticated leafy green vegetable in the Maya region of Guatemala, Belize, South-East Mexico during pre-Cambrian period [6] and due to its ease of cultivation and potential productivity, the plant has spread all over the world including the tropic. The leaves and shoot are taken as a laxative, diuretic, circulatory stimulants, to stimulate lactation and to harden fingernails [7]. Previous studies revealed that the plant leaves contain tannins, saponins, cardiac glycosides, deoxy sugar, and terpenes [6]. The leaves are attached to the stem by the petiole as single leaves.

The aerial part of the plant is usually made up of 3 or 4 leaflets held by one petiole. It has a root system that spreads and it grows well in aerated soil having a moderate amount of moisture and oxygen. It grows well within warm temperature ranges and is supported by soil possessing an adequate amount of nutrients.

The genus *Justicia*, named after the 18th-century Scottish botanist James Justice, belongs to the large family of Acanthaceae consisting of about 600 species of herbs and shrubs native to the tropics and subtropics [8, 9]. *J. carnea* is a flowering plant, widely distributed in various parts of Africa. In Nigeria, the shrubs of *J. carnea* are grown around homesteads and act as fences, which are easy to grow and propagate from stem cuttings by pushing the stems 1 to 2 inches into the soil [9]. A survey among the Igbo local populace in Nigeria revealed that the plant under study is locally called “ogwu obara” meaning blood tonic. The deep purple-colored juice from the leaves of this plant is extracted either by soaking or boiling in water, which can be drunk as tea. In other localities in Nigeria, the raw leaves are chewed and used together with “ogwu iba” as culinary vegetables to garnish yam porridge.

2. Material and methods

2.1. Collection and Identification of Specimens

Leaves of *C. aconitifolius* and *J. carnea*, were obtained from Rumuogba, Obio/Akpor Local Government of Rivers State. They were identified by Dr. Ekeke Chimezie of the Department of Plant Science Herbarium, University of Port Harcourt, and given the Voucher numbers UPH/V/1448 and UPH/V/1449 respectively.

This study was carried out in two phases, each lasting for 28 days. The first 28 days were used to induce lymphoma in the rats while the second phase (another 28 days), served as a treatment period using the extracts of *J. carnea* and *C. aconitifolius*. Seventy adult male Wistar rats were randomly divided into five (5) groups; two (2) control groups and three (3) treatment groups. The rats in group 1, received only food and water and served as the positive control. In group 2, the rats were induced with chloramphenicol and used as the negative control group. The treatment groups were administered with graded doses of *J. carnea* and *C. aconitifolius* aqueous extracts of 500, 1000, 1500, and 2000mg/kg weight respectively. A combined mixture of the *J. carnea* and *C. aconitifolius* extracts was administered in the same graded dose stated above. The animals were fed orally once daily for the entire duration using rat oral canula. The animals were anesthetized with ether after which blood was collected from the retro orbital venous plexus for determination of the hematological parameters. EDTA bottles were used to collect blood samples and were used to determine the hematological indices immediately. The percentage packed cell volume was determined using an automated hemoanalyzer. White blood cell counts (WBCs) were estimated using an automated hemoanalyzer. The method described by Osim *et al* [10] was used to carry out differential white cell count while blood collected in lithium heparin bottles were separated to plasma and used to assay for activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alkaline phosphatase (ALP) by the methods of (Reitman and Frankel, [11] urea and creatinine level by the method of Burtis *et al.*, [12] as outlined in Randox kits, UK. Carcinoembryonic antigen (CEA) was measured using enzyme-linked immunosorbent assay (ELISA), while C-reactive protein was measured using turbidimetric immunoassay.

2.2. Data Analysis

The data obtained from the experiment were expressed as mean \pm SEM. One-way analysis of variance (ANOVA) was used to analyze the data. Multiple comparisons were done using the post-Hoc test. The values were considered to be statistically significant at $p < 0.05$ [13].

3. Results and discussion

3.1. Effects of Aqueous Extracts of *J. carnea* and *C. aconitifolius* Leaves on some Haematological Indices of Wistar Rat

The results of the effect of the plant extract on some hematological indices are presented in Table 1. The effect of different concentrations of the plant extract on PCV showed a significant increase ($p < 0.05$) for the treated groups (3 - 14) when compared to the negative control. Relative to the positive control, the PCV level in the negative control was significantly ($p < 0.05$) lowered. The PCV levels for the treated groups were similar ($p > 0.05$) to the value for the positive control. The RBC for the positive control was 7.39 ± 0.54 while that of the negative control was 5.47 ± 0.22 . Similarly, there was an increase ($p > 0.05$) in RBC of the treated groups when compared to the negative control.

In this study, the hemoglobin concentration for the positive control was 14.34 ± 0.38 and the value for the negative control was 12.48 ± 0.51 . Comparing the hemoglobin concentration of the treated groups to the negative control revealed a dose-dependent significant increase ($p < 0.05$). The hemoglobin concentration of the positive control was significantly ($p < 0.05$) increased relative to the negative control, according to doses and plant extracts. The hemoglobin concentration for the treated groups was similar ($p > 0.05$) to the hemoglobin concentration of the positive control. The results of the WBC count in the control and extract treated groups were relatively ($p < 0.05$) lower compared to the negative control.

The total platelet levels for the positive and negative controls were 292.60 ± 56.93 and 238.80 ± 53.94 respectively. This showed a significant reduction ($p < 0.05$) in total platelet count in the blood field of rats not treated however, the positive control group and the groups treated with combined aqueous leaf extracts increased ($p < 0.05$) the platelets levels when compared to the negative control.

The liver marker enzymes assayed after 28 days of treatment showed a significant increase ($p < 0.05$) in the AST, ALT, and ALP concentration when compared with the untreated groups as seen in Table 2. There were significant decreases ($p < 0.05$) in the ALP and AST concentrations of rats in the treated groups when compared to group 2, the negative control that had a visible elevation of values in comparison with the positive control group.

The effect on cancer markers of the plant extracts on lymphoma-induced rats is shown in Table 3. There was a significant increase in the values of the C-reactive proteins and CEA respectively for the negative control. However, the combined extracts of *J. carnea* and *C. aconitifolius* significantly reduced the levels of CRP. While individual extracts and the combined extracts of *J. carnea* and *C. aconitifolius* significantly reduced the levels CEA.

3.2. Histopathology

Photomicrographs of blood films from Group 2 were compared to the normal control group, the presence of blast lymphocytes with other blood cell components appearing abnormal from the central structure were observed in Group 2 films. Similarly, the lymphocytes have microvesicular steatosis and an abnormal increase in size which is indicative of disruption of the architectural integrity of the blood cells of the rats due to the administration of chloramphenicol leading to blood cellular necrosis.

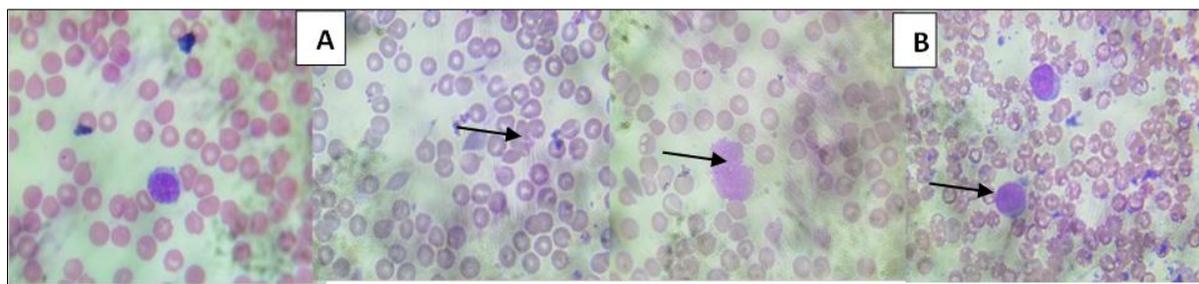


Figure 1 A - Photomicrograph of blood cells from Group 1 showing the normal presentation of hematological cells. Red blood cells, lymphocytes, and platelets appeared normal with even distribution. B - Blood cells from Group 2, showing the presence of blast lymphocytes with other blood cell components appearing abnormal from the central structure, lymphocytes have microvesicular steatosis and abnormal increase in size. [X100]

Table 1 Effect of aqueous extracts of *J. carnea* and *C. aconitifolius* blood profile of chloramphenicol-induced lymphoma in Wistar rat

| GROUP | PCV % | HGB g/dl | WBC 10 ³ /ul | PLT 10 ³ /ul | NEU 10 ³ /ul | LYMP 10 ³ /ul | MON 10 ³ /ul | EOS 10 ³ /ul | RBC 10 ³ /ul |
|--------|-------------------------|--------------------------|----------------------------|----------------------------|----------------------------|-----------------------------|----------------------------|----------------------------|----------------------------|
| GRP 1 | 41.50±2.50 ^a | 14.34±0.38 ^b | 10.30±0.38 ^a | 292.60±56.93 ^a | 43.00±2.00 ^b | 51.32±1.00 ^a | 4.50±0.92 ^a | 1.50±0.70 ^a | 7.39±0.54 ^a |
| GRP 2 | 39.02±2.50 ^b | 12.48±0.51 ^c | 12.40±0.51 ^b | 238.80±53.94 ^a | 24.00±0.90 ^a | 71.39±1.00 ^b | 3.50±0.40 ^a | 1.50±0.70 ^a | 5.47±0.22 ^b |
| GRP 3 | 42.00±2.00 ^a | 16.24±0.93 ^a | 5.50±0.30 ^b | 392.60±34.68 ^b | 26.50±1.36 ^b | 66.00±0.59 ^c | 6.50±0.61 ^a | 1.00±0.00 ^b | 7.43±0.37 ^a |
| GRP 4 | 41.00±1.00 ^a | 13.54±0.64 ^b | 11.10±0.64 ^c | 519.60±42.29 ^c | 35.00±2.04 ^b | 62.50±1.40 ^c | 2.00±1.69 ^b | 0.50±0.70 ^c | 6.34±0.52 ^a |
| GRP 5 | 45.00±5.00 ^b | 13.02±0.71 ^b | 11.02±0.71 ^c | 456.80±58.08 ^c | 26.50±1.35 ^b | 67.00±0.67 ^c | 4.00±1.13 ^b | 0.50±0.50 ^c | 5.72±0.34 ^b |
| GRP 6 | 41.00±1.00 ^c | 13.96±0.54 ^b | 11.96±0.54 ^c | 223.40±32.91 ^a | 35.00±0.00 ^a | 60.91±1.30 ^c | 3.50±1.44 ^b | 1.50±0.50 ^a | 6.56±0.22 ^a |
| GRP 7 | 44.50±1.50 ^a | 13.14±0.70 ^b | 11.14±0.70 ^a | 369.40±33.39 ^b | 28.50±0.83 ^a | 64.50±0.63 ^b | 5.50±0.59 ^c | 1.50±0.50 ^b | 5.78±0.33 ^b |
| GRP 8 | 43.50±3.50 ^d | 14.92±1.07 ^b | 5.56±0.41 ^d | 399.60±54.68 ^b | 46.50±2.18 ^a | 49.50±0.37 ^a | 2.00±0.68 ^c | 2.50±0.50 ^b | 6.18±1.03 ^a |
| GRP 9 | 43.00±7.00 ^e | 19.00±1.07 ^d | 10.50±3.52 ^e | 500.20±85.07 ^c | 42.00±1.71 ^b | 52.50±0.52 ^b | 3.50±0.54 ^a | 2.00±82.07 ^c | 8.64±0.72 ^c |
| GRP 10 | 49.50±0.50 ^e | 16.26±0.94 ^a | 11.74±2.80 ^e | 395.60±73.85 ^b | 34.50±1.51 ^b | 56.00±0.45 ^b | 8.00±0.33 ^c | 1.50±0.70 ^b | 9.42±0.64 ^c |
| GRP 11 | 45.50±2.50 ^a | 13.64±0.39 ^b | 11.00±0.94 ^a | 298.60±68.37 ^a | 26.00±0.40 ^d | 70.50±0.57 ^b | 3.00±0.23 ^a | 0.50±0.70 ^a | 8.59±0.66 ^c |
| GRP 12 | 48.50±2.50 ^f | 14.92±1.017 ^b | 10.92±0.17 ^f | 384.00±110.15 ^b | 26.5±0.84 ^a | 67.00±0.52 ^c | 3.50±0.41 ^b | 3.00±1.40 ^b | 6.96±0.46 ^a |
| GRP 13 | 42.00±7.00 ^a | 16.28±0.24 ^a | 11.28±0.24 ^a | 586.40±74.86 ^c | 32.50±1.56 ^a | 58.50±4.80 ^a | 5.00±0.38 ^a | 4.00±1.40 ^c | 5.20±0.60 ^b |
| GRP 14 | 46.50±4.50 ^f | 15.12±0.50 ^a | 10.12±0.50 ^a | 395.00±42.00 ^b | 29.50±0.34 ^a | 64.50±0.52 ^b | 2.50±0.70 ^c | 3.00±0.00 ^b | 6.87±0.22 ^a |

Data are expressed as mean± standard error mean (SEM) of n=5. Values in the same column having the same alphabet superscript are termed not to be statistically significant with each other at 0.05 significant level.

Table 2 Effect of aqueous extracts of *J. carnea* and *C. aconitifolius* on Liver Enzyme markers of chloramphenicol induced lymphoma Rats

| Groups | ALP IU/L | ALT IU/L | AST IU/L |
|--------|-------------------------|-------------------------|-------------------------|
| GRP 1 | 20.80±0.58 ^a | 18.60±1.08 ^a | 42.00±1.87 ^a |
| GRP 2 | 40.40±0.87 ^b | 31.60±0.93 ^b | 67.60±2.25 ^b |
| GRP 3 | 34.00±1.41 ^c | 30.00±1.00 ^b | 59.60±3.33 ^c |
| GRP 4 | 32.00±0.71 ^c | 34.40±1.81 ^c | 52.00±6.16 ^d |
| GRP 5 | 32.40±1.03 ^c | 37.80±1.24 ^d | 61.60±3.16 ^e |
| GRP 6 | 32.40±1.21 ^c | 26.20±1.98 ^e | 56.20±4.25 ^f |
| GRP 7 | 33.80±1.02 ^c | 37.20±3.87 ^d | 59.60±7.92 ^e |
| GRP 8 | 32.20±0.86 ^c | 33.80±2.08 ^c | 63.00±4.53 ^d |
| GRP 9 | 31.60±0.51 ^c | 28.60±1.44 ^e | 59.00±3.05 ^c |
| GRP 10 | 34.40±1.08 ^c | 36.60±2.62 ^d | 66.20±7.51 ^e |
| GRP 11 | 28.40±1.17 ^d | 33.20±2.22 ^c | 64.40±7.28 ^b |
| GRP 12 | 32.40±0.81 ^c | 26.20±3.93 ^e | 54.80±6.67 ^f |
| GRP 13 | 32.80±0.58 ^c | 37.40±1.44 ^d | 61.00±3.51 ^e |
| GRP 14 | 32.40±0.51 ^c | 29.80±1.69 ^b | 64.00±3.30 ^b |

Data are expressed as mean± standard error mean (SEM) of n=5. Values in the same column having the same alphabet superscript are termed not to be statistically significant with each other at 0.05 significant level.

Table 3 Effect of aqueous extracts of *J. carnea* and *C. aconitifolius* on Cancer markers of chloramphenicol induced lymphoma Rats

| Groups | CRP mg/ml | CEA ng/ml |
|--------|------------------------|------------------------|
| GRP 1 | 2.68±0.10 ^a | 1.18±0.16 ^a |
| GRP 2 | 3.24±0.31 ^b | 2.02±0.18 ^b |
| GRP 3 | 3.20±0.30 ^b | 1.92±0.26 ^b |
| GRP 4 | 3.10±0.35 ^b | 1.22±0.38 ^b |
| GRP 5 | 2.70±0.19 ^a | 1.38±0.32 ^c |
| GRP 6 | 3.02±0.18 ^b | 1.12±0.31 ^a |
| GRP 7 | 2.70±0.18 ^a | 1.30±0.27 ^b |
| GRP 8 | 2.18±0.29 ^b | 1.31±0.19 ^b |
| GRP 9 | 3.06±0.38 ^b | 1.32±0.19 ^b |
| GRP 10 | 3.14±0.32 ^b | 1.58±0.29 ^b |
| GRP 11 | 2.68±0.16 ^a | 1.62±0.43 ^c |
| GRP 12 | 2.34±0.31 ^b | 1.58±0.39 ^c |
| GRP 13 | 2.00±0.25 ^b | 1.28±0.17 ^a |
| GRP 14 | 2.96±0.23 ^b | 1.18±0.33 ^c |

Data are expressed as mean ± standard error mean (SEM) of n=5 values in the same column having the same alphabet superscript are term not to be statistically significant with each other at 0.05 significant level.

However, the photomicrographs of the extract-treated groups presented normal portal triadnormocytic, normochromic red blood cells with blast lymphocytes that disappeared progressively with the varying doses of the extracts and evenly distributed platelets. This impression is normal histology.

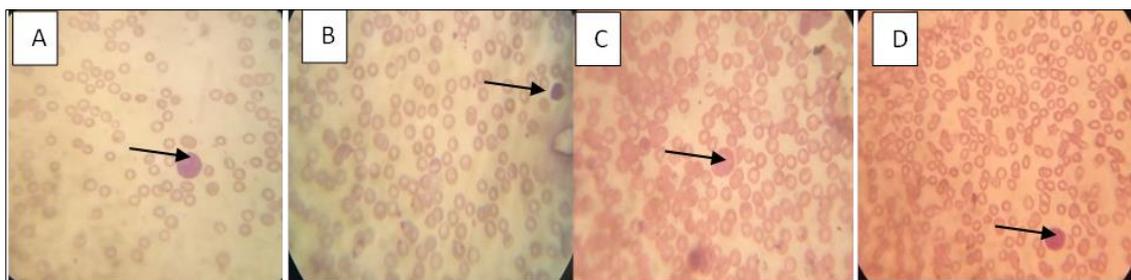


Figure 2 Photomicrograph of blood cells showing the presence of decreased lymphocytosis with all other hematological cells appearing normal. A: Group 3 (500 mg/kg body weight of *J. carnea*) showing normal hematological cell components and decreased lymphocytosis. B: Group 4 (1000 mg/kg body weight of *J. carnea*) showing hyper segmented neutrophil C: Group 5 (1500 mg/kg body weight of *J. carnea*) showing marked decrease in lymphocyte (Lymphocytosis) and blast lymphocytes D: Group 6 (2000 mg/kg body weight of *J. carnea*) showing corrected lymphocytes. [X100]

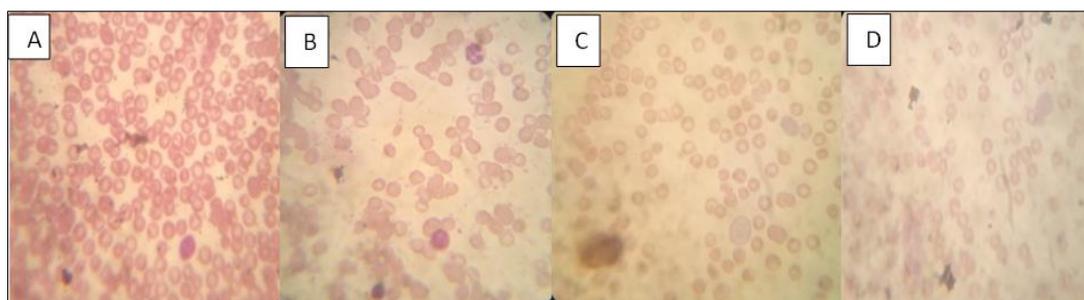


Figure 3 Photomicrograph of cellular components of blood, with the gradual disappearance of blast cells. A: Group 7 (500 mg/kg body weight of *C. aconitifolius*) showing normal cells with decreased lymphocytosis. B: Group 8 (1000 mg/kg body weight of *C. aconitifolius*) showing hyper segmented neutrophil. C: Group 9 (1500 mg/kg body weight of *C. aconitifolius*) showing a marked decrease of blast lymphocytes. D: Group 10 (2000 mg/kg body weight of *C. aconitifolius*) showing marked lymphocytosis. [X100]

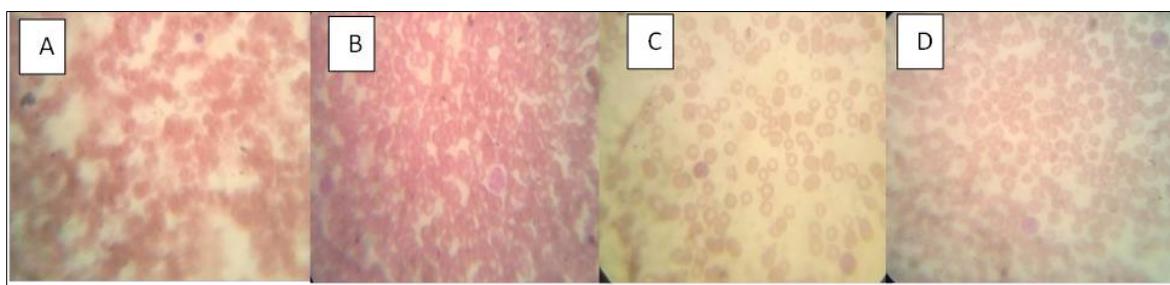


Figure 4 Photomicrograph of cellular components of blood. A: Group 11 (500 mg/kg body weight of *J. carnea* and *C. aconitifolius*) showing marked lymphocytosis. B: Group 12 (1000 mg/kg body weight of *J. carnea* and *C. aconitifolius*) showing hyper segmented neutrophil. C: Group 13 (1500 mg/kg body weight of *J. carnea* and *C. aconitifolius*) showing lymphocytes and hematological cells. D: Group 14 (2000 mg/kg body weight of *J. carnea* and *C. aconitifolius*) showing normal lymphocytes. [X100]

4. Discussion

Herbs are widely perceived by the general public as being free from side effects; however, herbal therapies have not been effusively researched or standardized to enable the clinical application. Morphological, Histopathological Hematological, and Biochemical effects after the use of some medicinal herbs have been reported in the works of [14, 15, 16, 17]. The present study examined the biochemical implications of chloramphenicol-induced lymphoma and the

ameliorative potentials of *J. carnea* and *C. aconitifolius* in male Wistar rats. Hematological, extracts of *J. carnea* and *C. aconitifolius* showed a significant effect on the hematological profile of Wistar rats. The packed cell volume (PCV) of animals in group 1 (positive control) with a value of 41.0 ± 2.50 was significantly different ($p < 0.05$) from group 2 (negative control) with a mean value of 39.02 ± 2.50 . In the treatment groups, there were also significant values when compared with both positive and negative control groups. Groups 10 and 12, which are aqueous extracts of *C. aconitifolius* (2000mg/kg) and a combined mixture of *J. carnea* and *C. aconitifolius* (1000mg/kg), showed the highest significant result at (49.50 ± 0.50) and 48.50 ± 2.50 respectively. The results obtained support the findings of Orjiakor [1], which posits that *Justicia carnea* has anti-anemic potentials. Hemoglobin did not have any significant difference in both positive and negative groups. However, groups 9 (1500mg/kg *C. aconitifolius*), 10 (2000mg/kg of *C. aconitifolius*), and 12 (1000mg/kg of a combined mixture of *J. carnea* and *C. aconitifolius*) showed significant increases that differ from the other groups. Lymphocytes were highly elevated as showed in the negative control group (group 2) with values 71.39 ± 1.00 when compared with 51.32 ± 1.00 in group 1. There was a general significant increase ($p < 0.05$) in the WBC count of rats in the treatment groups when compared to group 1, except groups 3 (500mg/kg *J. carnea*), group 12 (1000mg/kg of a combined mixture of extracts) and group 14 (2000mg/kg of a combined mixture of extracts) suggesting that the animals are still struggling with the effect of the disease state. The initial increase in the WBC count could be a result of lymphoma that was induced in the animals. The animal's immune system may assume that the cause of lymphoma could be a result of infection or disease and hence increase the production of white blood cells to fight such infections [1, 18]. It has been known that WBC counts increase rapidly following a foreign attack by pathogens on the system and the system's normal physiologic response will be to boost the body defense mechanisms [1, 19]. The various aqueous extracts of *J. carnea* and *C. aconitifolius* plants showed a significant decrease ($p < 0.05$) in the values of the treated groups when compared with group 2. Groups 8 (49.50 ± 0.37) had the most significant decrease in lymphocytosis of the treated group in comparison with the negative control group. Preliminary phytochemical analysis of *J. carnea* and *C. aconitifolius* shows the presence of alkaloids, flavonoids, glycosides, carbohydrates, saponins, tannins, terpenoids, phenols, steroids, and resins [20]. It posits that flavonoids are present in high concentrations. Flavonoids are the largest group of plant phenols and provide much flavor and color to fruits and vegetables [1, 21]. This may be attributed to the strong flavor and deep red color despite the green leaf of *J. carnea* when boiled as ingested. Flavonoids and phenols constitute a wide range of substances that play important roles in protecting biological systems, serve as potent water-soluble antioxidants and free radical scavengers which prevent oxidative cell damage, lower the risk of heart diseases, and have strong anticancer activity [22]. Hence, the aqueous leaf extract of *J. carnea* and *C. aconitifolius*, possess some biologically active compounds which could serve as a potential source of drugs and preventive measures against oxidative damage when ingested by animals. Figures 1-4 showed the blood films of the chloramphenicol-induced lymphoma in Wistar rat and the effect of aqueous extracts of *J. carnea* and *C. aconitifolius* leaves. Figure 1A is blood films of the positive control group with all cells intact; red blood cells (RBC), platelets, Lymphocytes, eosinophils, appearing normal. In Figure 1B (negative control group), the red blood cell integrity was maintained but multiple blasts (or lymphocytosis) were seen. The multiple blasts and general elevation of the white blood cells (WBCs) are indicators of lymphoma. Similarly, Figures 4A, B, C and D are blood films with different doses of aqueous extract of *J. carnea* and *C. aconitifolius*. The films showed a dose-dependent effect on the blood film, with the gradual disappearance of the lymphocytosis. This was most visible in Figures 4C and D, the 1500mg/kg and 2000mg/kg of the mixture of *J. carnea* and *C. aconitifolius*.

There were significant decreases ($p < 0.05$) in the ALP and AST concentrations of rats in the treated groups when compared to Group 2, the negative control that had a visible elevation of values in comparison with the positive control group. The decreases in the activities of the assayed liver enzymes at different doses of the extracts per body weight administered could suggest that the extract is not hepatotoxic. Studies have shown that persistent elevation of serum ALT, AST, and ALP levels are reliable markers for hepatotoxicity [23]. The result of the liver enzyme markers showed that the aqueous leaf extract of *J. carnea* and *C. aconitifolius*, do not have any adverse effect on the hepatocytes. The leaves are rich in phenols and flavonoids and studies have shown that phenols and flavonoids can inhibit xenobiotic-induced hepatotoxicity in experimental animal models due to their potent antioxidant or free radical scavenging activities [24].

The combined extracts of *J. carnea* and *C. aconitifolius* significantly reduced the levels of CRP. While individual extracts and the combined extracts of *J. carnea* and *C. aconitifolius* significantly reduced the levels CEA. A high level of CRP has been associated with chronic inflammatory reactions, high risk of cardiovascular disease, and high risk of lung and colon cancers. The concentration of C-reactive protein present in the serum of experimental animals is proportional to the duration of exposure of the animals to chloramphenicol. There is a correlation between the towering amount of C-Reactive protein and the risk of developing cancer [25].

5. Conclusion

The investigation of the biochemical implication of chloramphenicol-induced lymphoma and the ameliorative potential of aqueous extracts of *J. carnea* and *C. aconitifolius* in male Wistar rats showed that the extracts possessed anti-anemic activity as revealed by the significant increase in the hematological profile. The extracts also showed hematopoietic potential, increasing the levels of hemoglobin, packed cell volume, and white blood cells. Its effect in the lowering of the multiple blasts of lymphocytes (lymphocytosis) was demonstrated. These further established the blood-boosting and anti-cancer potential of the plant extract considering that lymphocytosis is closely is often implicated in acute blood diseases and blood cancer. The evaluation of the ameliorative potentials of the extracts of *J. carnea* and *C. aconitifolius*, revealed that the extract had a significant impact on the induced animals. This conclusion is drawn from the observed increase in the hematological profile of all extract-treated groups.

The ameliorative effects of *J. carnea* and *C. aconitifolius* might therefore be as a result of any one or a combination of the phytochemicals present in the plants. The use of the plant material in folkloric medicine is thus verified by this study; in addition, the blood-boosting property observed imparts an advantage to the plant's use as a traditional blood supplement.

Compliance with ethical standards

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Disclosure of conflict of interest

The Authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Statement of ethical approval

As per international standard or University standard ethical approval has been collected and preserved by the authors.

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