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Original Research Article

Annona muricata Leaf Extract Modulates Chloramphenicol-Induced Lymphoma in Female Wistar Rats

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ABSTRACT

Chloramphenicol, an antibiotic used to treat bacterial infections, is also implicated in several conditions, including aplastic anaemia that progresses to leukaemia. The modulatory potential of leaf extract of *Annona muricata* on chloramphenicol-induced lymphoma (CIL) in female Wistar rats was investigated. Forty-eight adult female rats with an average weight of 186 g were randomized into 6 groups of 8 rats each. Rats in Group 1 served as the positive control. Each rat in Groups 2-6 was orally administered with 250 mg/kg bodyweight chloramphenicol once daily for 28 days. The rats were given access to basal diet and water. After 28 days, blood-film analyses were carried out from each of the groups. The presence of blast and lymphoproliferative cells in Groups 2-6 confirmed CIL. Groups 1 and 2 rats received distilled water, while Groups 3-6 received 500, 1000, 1500, and 2000 mg/kg, respectively, of *A. muricata* for another 28 days. The animals were anesthetized, blood was collected from the retro-orbital venous plexus for determination of haematological indices; oxidative stress markers; lactate dehydrogenase, superoxide dismutase, catalase, glutathione, malondialdehyde, cardiac markers; Troponin-1, myoglobin, cancer indices; C-reactive protein, carcinoembryonic antigen, Alpha-fetoprotein. There was significant (p<0.05) amelioration of impaired haematological indices in the treated groups when compared to Group 2. Troponin-I and myoglobin were significantly (p<0.05) lowered in the treated groups when compared to Group 2. Oxidative stress markers and cancer indices were increased in Group 2, but significantly (p<0.05) ameliorated in Groups 3-6. Therefore, *A. muricata* has the potential to modulate adverse effects of CIL.

Keywords: *Annona muricata*, Chloramphenicol-induced lymphoma, lymphoproliferative, Haematological indices, Histopathology

Introduction

Leukaemia is a group of blood cancers that usually begin in the bone marrow and characterized by an abnormal increase of immature white blood cells called blasts or leukaemia cells.[1] It usually involves white blood cells which are known to be potent infection fighters that grow normally and differentiate in an orderly way as the body needs them. However, in people with leukaemia, the bone marrow produces abnormal white blood cells, which do not function properly. Symptoms may include bleeding and bruising, feeling tired, fever, and an increased risk of infection. These symptoms occur due to the lack of normal blood cells.[1] There are four main types of leukaemia; Acute myeloid leukaemia (AML), Acute lymphoblastic leukaemia (ALL), Chronic lymphocytic leukaemia (CLL) and Chronic myeloid leukaemia (CML) as well as a number of less common types.[2,3] Leukaemia and lymphomas both belonging to a broader group of tumours that affect the blood, bone marrow, and lymphoid system are known as tumours of the hematopoietic and lymphoid tissues.[4] In 2015, leukaemia was present in 2.3 million people and caused 353,500 deaths worldwide.[5] In 2020, it newly developed in 474,519 people with over 311,594 deaths.[2]

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It is the most common type of cancer in children with three-quarters of leukaemia cases being the Acute lymphoblastic type. The most common symptoms in children are easy bruising, pale skin, fever, and an enlarged spleen or liver.[1] Treatment may involve some combination of chemotherapy, antibiotics, blood transfusion, radiation therapy, targeted therapy, and bone marrow transplant in addition to supportive care and palliative care.[1] The success of treatment depends on the type of leukaemia and the age of the person. Outcomes have improved in the developed world,[3] the average five-year survival rate is 57% in the United States. In children under 15 years of age, the five-year survival rate is greater than 60 to 85%, depending on the type of leukaemia. In children with acute leukaemia who are cancer-free after five years, the cancer is unlikely to return.[6] Although these treatments have prolonged the survival rates of patients with leukaemia, some of these treatments are difficult to handle.[7] The exact cause of leukaemia is unknown. A combination of genetic factors and environmental (non-inherited) factors are believed to play a role.[8] Furthermore, several factors have been identified which may increase the risk of having leukaemia, and they include: a family history of leukaemia; smoking, which increases the risk of developing AML; genetic disorders such as Down syndrome; blood disorders such as myelodysplastic syndrome, which is sometimes called pre-leukaemia; previous treatment of cancer with chemotherapy or radiation; exposure to high levels of radiation; exposure to chemicals such as benzene.[9]

Many reports have proven the efficacy of chloramphenicol in the treatment of bacterial infections. It is a popular antibiotic used as an eye ointment to treat conjunctivitis, also for the treatment of typhoid fever, and other dangerous infections where other antibiotics are not effective.[10] However, this drug with high beneficial activity is also associated with numerous side effects. Some of these common side effects may include but not limited to; insufficient or low production of red blood cells (aplastic anaemia), bone marrow suppression, diarrhoea,

and enterocolitis. Accumulation of chloramphenicol may increase the risk of gray syndrome, especially in premature infants and neonates. This may be due to decreased hepatic and renal function as a result of chloramphenicol exposure.[11-14] According to Drugs.com,[15] chloramphenicol-associated aplastic anaemia (terminating in leukaemia) has been reported. Serious and fatal blood dyscrasias (aplastic anaemia, hypoplastic anaemia, thrombocytopenia, granulocytopaenia) can occur after short-term or prolonged therapy with chloramphenicol. Other side effects associated with chloramphenicol treatment are headache, nausea, nightmares, inflammation of the optic nerve, weakness and numbness in the hands and feet, rash, inflamed and sore mouth, vomiting and leukaemia amongst others.[16] Its usage has notably led to several adverse health effects which on several instances remain undiagnosed to a later but terminal periods. These have triggered research into the possible causative mechanisms behind the inducement of leukaemia from the intake of chloramphenicol and a potential therapy for these side effects.

The study of using natural extracts as medications or health-improving agents is known as phytotherapy. It is a crucial component of the conventional medical system and is frequently used in conjunction with conventional therapy to treat stubborn ailments including cancer.[17,18,19] Extracts from *A. muricata* are among a myriad of botanical products which have promising medicinal values. Numerous health benefits[20] have been attributed to *A. muricata*, most of which has been linked to its antioxidant potential.[21,22] This study was carried out to ascertain the effect of prolonged dose of chloramphenicol on the erythropoietic processes and its cytotoxic potential in inducing leukaemia and the modulatory prospective of aqueous leaf extract of *A. muricata* on CIL in female Wistar rats.

Materials and Methods

Collection and identification of specimens

Fresh leaves of *A. muricata* were collected in January, 2021 from Choba village, Rivers State, Nigeria. The plant was authenticated by Dr. Ekeke Chimezie of the Department of Plant Science and Biotechnology Herbarium, University of Port Harcourt, and given the voucher number UPH/V/1447. The leaves were washed with distilled water and air-dried at room temperature (29±1°C) for 3 weeks and then pulverized with the aid of Marlex Excellent grinder (Mumbai, India). The ground samples were then passed through a sieve of 0.5 mm pore size to obtain a fine uniform powder.[21] The powdered sample (300 g) was soaked in 650 ml distilled water and allowed to macerate at room temperature for 72 hours. The suspension was filtered through Whatman filter paper No 4 and dried in water bath at 55°C. The dried extract was weighed and stored in a clean reagent bottle, then preserved in a refrigerator at 4^{O}C before use.[22]

Experimental design

Forty-eight adult female Wistar rats with an average weight of 186 g were bought from animal breeders and kept in the Department of Biochemistry, University of Port Harcourt Animal house. The rats were allowed to get acclimatized to the environment for one week and had unrestricted access to normal rat chow and water *ad libitum*. They were housed in plastic cages with dry sawdust shavings as bedding material under standard conditions of temperature (23 ± 1°C), humidity (50-60%), and 12-hour light/12-hour dark cycle throughout the 56-day study period. The animals were handled in accordance with the requirements of the National Institute of Health Guidelines for Animal Care and Use of Laboratory Animals (Publication Number, 85–23). The rats were randomly placed in 6 groups consisting of 8 rats each. Rats in group 1 received 0.9% physiological saline orally and served as the positive control. Each rat in groups 2-6 was orally administered with 250 mg/kg body weight chloramphenicol palmitate BP, Bombay, India, once daily for 28 days with the aid of rat cannula. The rats were closely observed during this period and free access to basal diet and water were maintained during the study. At the end of 28 days, haematogram and blood film analysis were made from each of the groups and the presence of blast lymphoma cells confirmed CIL.[15]

Administration of aqueous leaf extract of A. muricata

Upon confirmation of CIL in groups 2-6, rats in group 2 did not receive any treatment and served as the negative control, while rats in groups 3-6 received 500, 1000, 1500, and 2000 mg/kg body weight of aqueous leaf extract of *A. muricata* respectively for another 28 days. All groups had access to feed and water. The Department of Biochemistry, University of Port Harcourt Ethics Committee for research on Laboratory Animals gave its approval for the study protocols and design, as well as the methodology for handling the animals (UPH/BCHREC/2022/001B), and they were in accordance with the Standard Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication Number, 85-23).

Haematological and Biochemical studies

The animals were anaesthetized with ether after which blood was collected from the retro-orbital venous plexus into EDTA bottles for immediate determination of haematological indices. Full blood count was determined using an automated haemoanalyzer. The method described by Osim *et al*.[23] was used to carry out differential white cell count. The prepared slides were examined with a Motic™ compound light microscope using x4, x10, and x40 objective lenses and photomicrographs taken using a Motic™ 9.0 megapixels microscope camera. Blood for biochemical assays was collected in lithium heparin bottles, separated from plasma and used to assay for the activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT),[24] and alkaline phosphatase (ALP)[25] as outlined in Randox kits, UK. The measurement of sodium and potassium was done using a flame photometer as described by Chuang *et al*.[26] Urea and creatinine levels were measured by the method of Tietz.[27] Cardiac markers [troponin 1 (TP-1), myoglobin (MB)] and cancer indices [Carcinoembryonic antigen (CEA) and Alpha-fetoprotein (AFP)] were measured using enzyme-linked immunosorbent assay (ELISA), while C-reactive protein (CRP) was measured using turbidometric immunoassay. The methods of Usoh *et al*.[28] were adopted for the determination of the activities of oxidative stress markers such as lactate dehydrogenase (LDH), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and malondialdehyde (MDA).

Data analysis

The results obtained were expressed as mean ± standard deviation. Results were read in triplicates and were used for these calculations. Analysis of variance (for multiple comparisons) was used. Statistical Package for Social Science (SPSS) 22.0 of windows was used for the analysis. Results with p value < 0.05 were considered significant.

Results and Discussion

Haematological indices of CIL in female Wistar rats after treatment with graded doses of aqueous leaf extract of A. muricata

The results obtained from the acute toxicity study showed no fatality, thus the crude aqueous extract of *Annona muricata* was safe for consumption up to 5,000 mg/kg bodyweight.[29] The extracts exerted dose-dependent remedy in chloramphenicol-induced rats. There were significant (p<0.05) amelioration of haematological impairment in Hb, RBC, and WBC of treated groups when compared to the negative control group (Figure 1). The MCV and MCH values were not significant (p>0.05). A significant (p<0.05) increase in the platelets of rats administered 1000, 1500, and 2000 mg/kg aqueous extract was observed in comparison with rats in group 2.

With numerous reported *in vitro* and *in vivo* pharmacological properties, the aerial portions of *A. muricata* have undergone significant research and have proven to be successful in the treatment of a number of cancer types.[29,30] The nature of the phenolic chemicals present in soursop leaves may help to explain the protective capacity of the leaves. These compounds may be responsible for the antihaemolytic activity they exhibited since they not only stabilize free radicals but also make erythrocytes more resistant to oxidative stress.[31] The significant results of the modulatory effects of soursop leaf extract on the haematology indices were similar to those of Cyboran *et al*.,[32] who investigated changes in pig erythrocytes induced by extracts of currant, strawberry and apple leaves, and they showed that the polyphenolic compounds

present in the extracts had a positive effect on the erythrocytes. In a similar study, it was reported that aqueous leaf extracts of *Justicia carnea* and *Cnidoscolus aconitifolius* showed hematopoietic potential by increasing the levels of haemoglobin, packed cell volume, and white blood cells and may have an ameliorative effect in blood diseases connected to over-exposure to chloramphenicol.[33]

Liver enzymes of CIL in female Wistar rats treated with graded doses of aqueous leaf extract of A. muricata

Aqueous leaf extract of *A. muricata* significantly (p<0.05) inhibited hepatic disorder in rats (Figure 2). The extract dose-dependently and significantly (p<0.05) restored normal hepatic status with respect to ALP, ALT, and AST levels in rats.

The decrease in the activity of the assayed liver enzymes at different doses of the extract 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.[34] The result of the liver enzyme markers showed that the aqueous leaf extract of *A. muricata* does not harm the hepatocytes.[21] 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.[35]

Effect of aqueous leaf extract of A. muricata on kidney function parameters of CIL in female Wistar rats

There is a non-significant (p>0.05) difference between the control group and treatment groups for sodium, urea and creatinine. However, the potassium concentration decreased significantly (p<0.05) in the treatment groups when compared with group 2, which served as the negative control (Figure 3).

Urea depends on intake and some other factors, hence, the increase could be attributed to the amino acid content of the drug (chloramphenicol). In assessing renal function, urea concentration depends on some parameters, so creatinine is more reliable since its rate of production is constant and the rate of excretion depends on the muscle mass of the animal involved.[33]

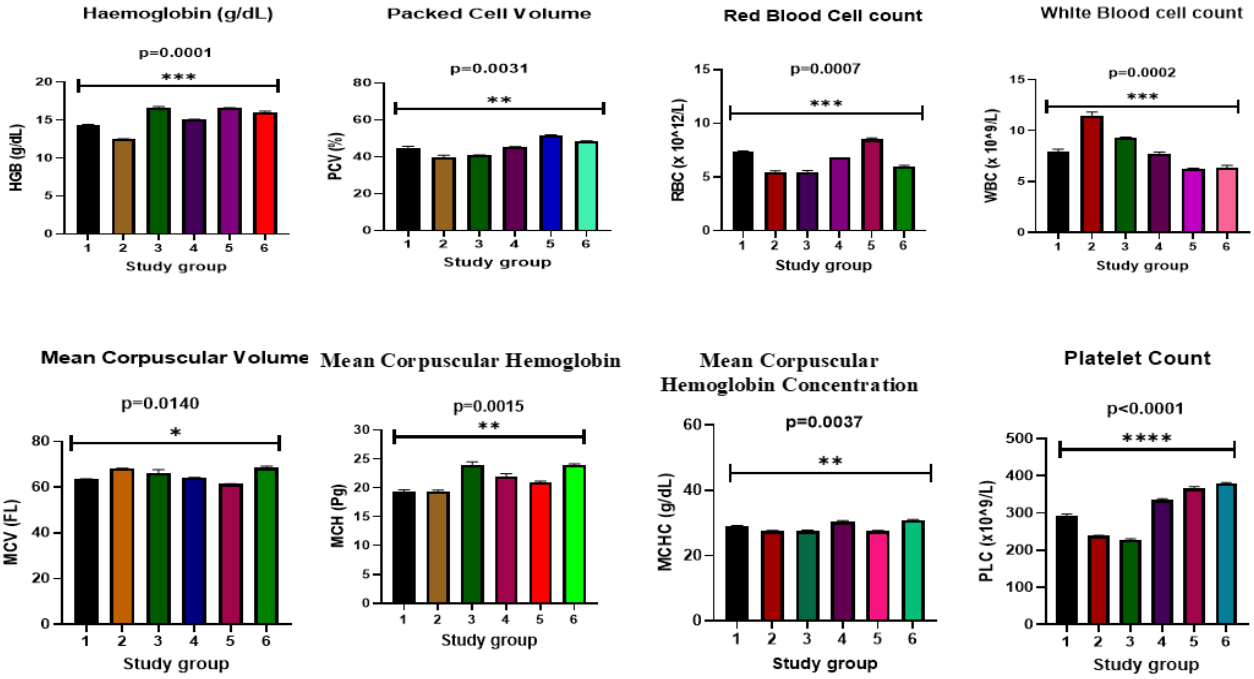

Figure 1: Haematological indices of CIL in female Wistar rats treated with graded doses of aqueous leaf extract of *A. muricata.* Data are mean ± SD (n = 8). n = number of rats in each group.

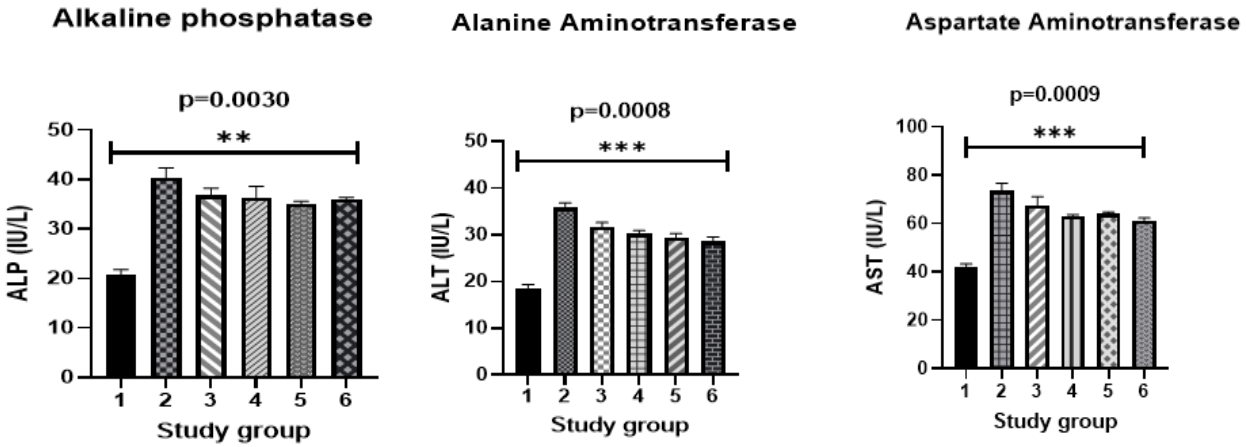

Figure 2: Liver enzymes of CIL in female Wistar rats treated with graded doses of aqueous leaf extract of *A. muricata.* Data are mean ± SD (n = 8). n = number of rats in each group.

Effect of aqueous leaf extract of A. muricata on cardiac and cancer markers of CIL in female Wistar rats

Cardiac makers (TP-I and MB) were significantly lowered (p<0.05) in the treatment groups when compared to the negative control (Figure 4). Similarly, the cancer markers such as, CRP and CEA were decreased in the groups treated with aqueous leaf extract of *A. muricata* while AFP was significantly increased (p<0.05) in group 2 when compared with group 1. However, groups 3-6 showed more significant ameliorative potential with a dose-dependent significance that shows a possibility of the *A. muricata* extract to remedy lymphoma.

Results of cardiac markers (TP-I and MB) and cancer markers (CRP, CEA, and AFP) evaluated in this study were very significant and suggests that the chloramphenicol administered did not only induce lymphoma in the blood but also affected the physiological function of the heart. CRP is a simple and effective prognostic marker in patients with acute myeloid leukaemia[36] and has been reported to be a prognostic indicator in a variety of haematologic malignancies[37] and solid tumours.[38] Its elevations in the plasma has been associated with shorter survival and development of second cancer in patients with chronic lymphocytic leukaemia.[39] Evidence from clinical trials with several types of cancer and trastuzumab, an anticancer drug, has shown an increase in cardiac troponin levels corresponding to myocardial dysfunction. Simoes *et al*.[40] have reported an increase in cardiac troponin levels in breast cancer patients. Also, Zafar *et al*.[41] reported an increase in troponin as well as myoglobin in acute myocardial infarction. Myoglobin is a muscle-associated respiratory protein expressed in multiple types of cancer, including breast and prostate tumours. Elevated levels of MB have also been associated with epithelial cancers.[42] Similarly, higher CEA levels have been reported in 64% of children at the onset of acute leukaemia and during bone marrow relapse. However, AFP values in different stages of the disease were sporadically elevated but did not reflect the activity of leukaemia.[43] This study suggests that TP-1 and MB, although cardiac markers, CEA and CRP may be elevated in the presence of lymphoma or leukaemia and that *A. muricata* has the potential to modulate the cytotoxic effect of chloramphenicol-induced lymphoma in female Wistar rats.

Oxidative stress markers of CIL in female Wistar rats after treatment with aqueous leaf extract of A. muricata

MDA and LDH concentrations were dose-dependently and significantly (p<0.05) modulated by the aqueous leaf extract of *A. muricata* (Figure 5). CAT activity in groups 1 – 4 did not change significantly, however, there was a significant increase in the CAT activities of groups 5 and 6 when compared to the control and untreated groups. GSH and SOD showed a marked uncharacteristic difference in the concentrations between the negative control and groups 4, 5, and 6.

Analysis of blood parameters indicates multiple blasts of haematological cells which can be alleviated by antioxidants especially from natural sources such as *A. muricata*. The aqueous leaf extract exhibited much inhibition of cytotoxicity and improved blood parameters. This tends to suggest that the aqueous leaf extract of *A. muricata* may be an inhibitor of reactive oxygen species generation and may possess strong antioxidant properties and could serve as a potent remedy for the control of anaemia and CIL. Several investigations on the antioxidant properties of the leaf extract of *A. muricata* have been reported.[44-47] Moreover, flavonoids present in *A. muricata* leaves could account for the protection of cell membranes because they interrupt the interaction of phospholipid components and inhibit their oxidation, thereby protecting them from damage caused by oxidizing molecules.[48]

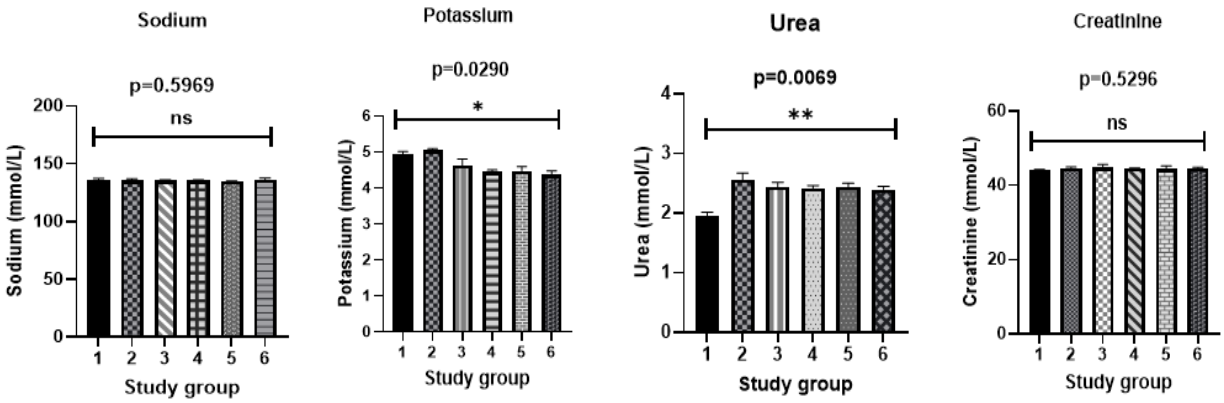

Figure 3: Effect of aqueous leaf extract of *A. muricata* on kidney function parameters of CIL in female Wistar rats. Data are mean ± SD (n = 8). n = number of rats in each group.

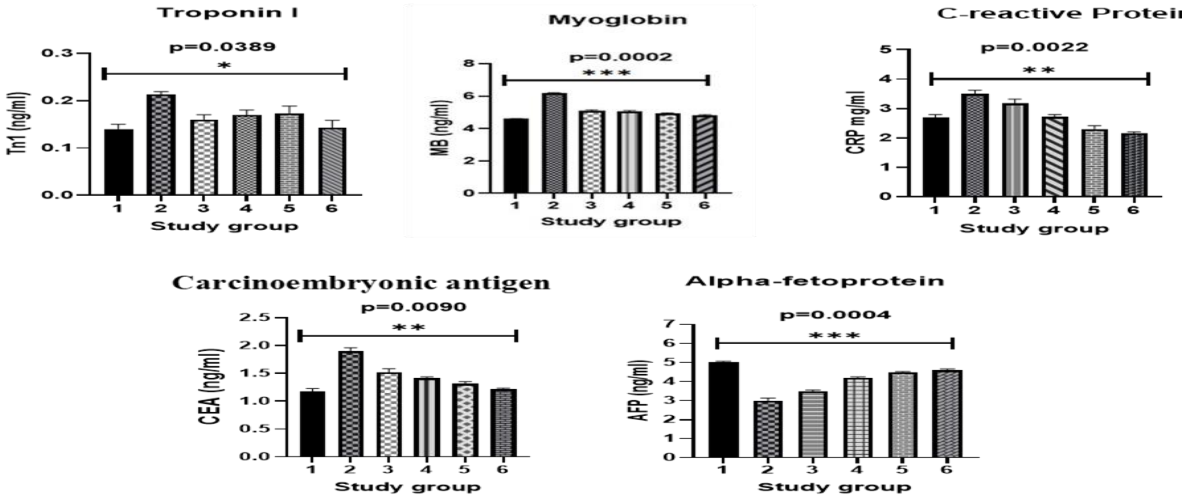

Figure 4: Effect of aqueous leaf extract of *A. muricata* on cardiac and cancer markers of CIL in female Wistar rats. Data are mean ± SD (n = 8). n = number of rats in each group.

Histopathological findings

Photomicrographs of red blood cells from study Groups 1 to 6 are presented in Plate 1 (A - F).

Plate A: Photomicrograph of red blood cells from group 1 with normocytic normochromic cells seen. Neutrophils are mature with 3 - 4 nuclear segmented forms giving an impression of essentially normal blood film.

Plate B: Photomicrograph of blood films of female Wistar rats that received 250 mg/kg chloramphenicol without any treatment (Group 2). The red blood cells appeared slightly reduced in number on the film and showed anisocytosis. The film presented a dimorphic picture of normocytic, normochromic, and numerous polychromatic cells. White blood cells appear adequately in number to film. A few lymphoplasmacytoid cells are present showing clumped nuclear chromatin, basophilic cytoplasm, and a perinuclear halo. A few atypical forms are present on the film showing a cleaved/folded nuclear chromatin patterns. Some lymphoblasts are also present on the film showing large forms with loose nuclear chromatin and 1-2 nucleoli. Neutrophils are mature with some showing hyper-segmented forms having up to 9 nuclear segments. Cytoplasmic granules show primary and secondary granules (normal granulation). Monocytes are present and mature. Platelets appear adequately in number on the film with normal granular pattern. The impression is a typical lymphocytosis and lymphoproliferative neoplasm secondary to Acute lymphoblastic leukemia

Plate C: Photomicrograph of blood films of female Wistar rats that received 250 mg/kg chloramphenicol and treated with 500 mg/kg bodyweight of *A. muricata* (Group 3). Red blood cells appear scantily distributed on the film and show a dimorphic picture of normocytic, normochromic, and polychromatic cells. There is lymphocytosis, the white blood cells appear slightly increased in number on the film with a predominance of mononuclear cells. There is relative lymphocytosis with numerous atypical lymphocytes present on the film showing a loose nuclear chromatin pattern with cleaved nuclei, folded nuclei, and very few nucleoli. Numerous smudge cells are also present on film. Neutrophils are mature with some showing hyper-segmented forms having up to 9 nuclear segments. Cytoplasmic granules show primary and secondary granules (normal granulation). Monocytes are present and mature. Platelets appear reduced in number on the film with normal granular pattern. The impression is a typical lymphocyte/lymphoblast secondary to lymphoproliferative neoplasm.

Plate D: Photomicrograph of blood films of female Wistar rats that received 250 mg/kg chloramphenicol and treated with 1000 mg/kg bodyweight of *A. muricata* (Group 4). Red blood cells appear adequately on film and show normocytic normochromic red cells. White blood cells appear adequate in number to the film with a predominance of mononuclear cells. There is relative lymphocytosis with mostly small and mature forms present showing clumped nuclear chromatin and a thin rim of cytoplasm. A few reactive lymphocytes and lymphoplasmacytoid forms are seen on film. Neutrophils are mature with some showing hyper-segmented forms having up to 7 nuclear segments. Cytoplasmic granules show primary and secondary granules. Monocytes are present and mature. Platelets appear reduced in number on the film with normal granular pattern. The impression is reactive lymphocytes secondary to a chronic inflammatory reaction.

Plate E: Photomicrograph of blood films of female Wistar rats that received 250 mg/kg chloramphenicol and treated with 1500 mg/kg bodyweight of *A. muricata* (Group 5). Red blood shows a dimorphic picture of normocytic, normochromic, and numerous polychromatic cells. White blood cells appear adequate in number to film with a predominance of mononuclear cells. There is relative lymphocytosis with numerous lymphoblast present on the film showing a loose nuclear chromatin pattern with 1-2 nucleoli. Few smudge cells and small mature lymphocytes are also present on film. Neutrophils are mature with some showing hyper-segmented forms having up to 8 nuclear segments. Cytoplasmic granules show primary and secondary granules (normal granulation). Monocytes are present and mature. Platelets appear adequately in number on the film with normal granular pattern. The impression is Lymphoproliferative Neoplasm.

Plate F: Photomicrograph of blood films of female Wistar rats that received 250 mg/kg chloramphenicol and treated with 2000 mg/kg bodyweight of *A. muricata* (Group 6). Red blood shows a dimorphic picture of normocytic, normochromic, and numerous polychromatic cells. White blood cells appear adequate in number to film with a predominance of mononuclear cells. There is relative lymphocytosis with some reactive forms present on the film showing abundant cytoplasm with clumped nuclear chromatin. A few show loose nuclear chromatin and cytoplasmic granules. Few smudge cells are also present on film. Neutrophils are mature with some showing hyper-segmented forms having up to 8 nuclear segments. Cytoplasmic granules show primary and secondary granules (normal granulation). Monocytes are present and mature. Platelets appear adequately in number on film with normal granulation. The impression is Reactive lymphocytes.

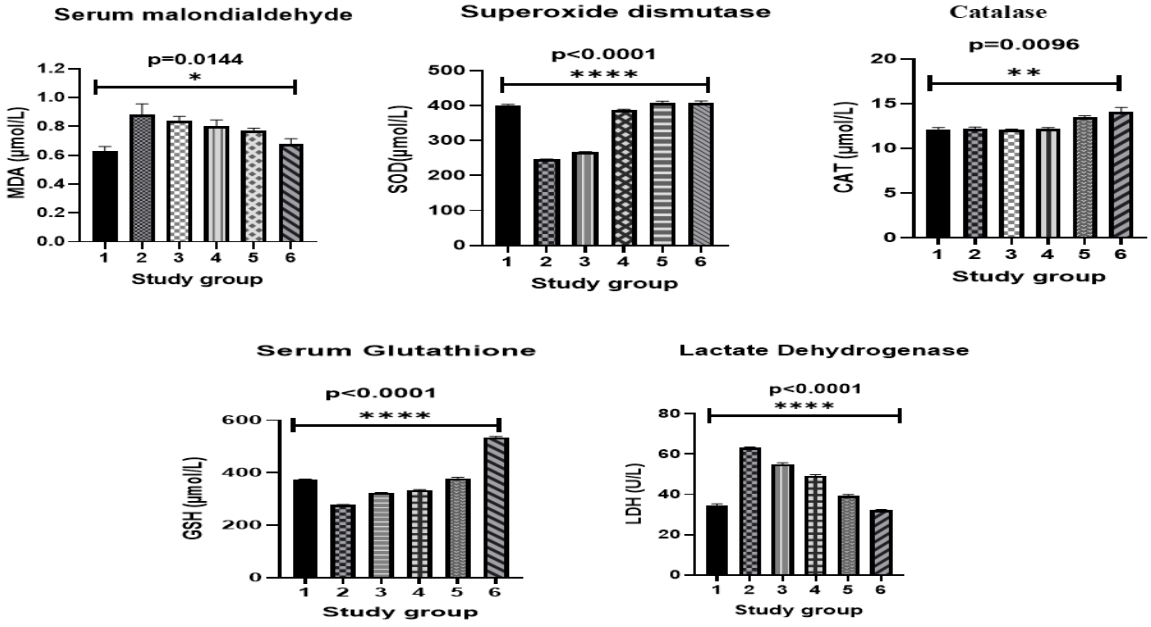

Figure 5: Effect of aqueous leaf extract of *A. muricata* on Oxidative stress markers of CIL in female Wistar rats. Data are mean ± SD (n = 8). n = number of rats in each group

The red blood cells in the photomicrographs of experimental animals that received 250 mg/kg of chloramphenicol without treatment (Plate B) appeared slightly reduced in number. They were normocytic, normochromic and had numerous polychromatic cells and showed anisocytosis. There were few lymphoplasmacytoid cells showing clumped nuclear chromatin, basophilic cytoplasm, and a perinuclear halo. Some lymphoblasts were also present on the film showing large forms with loose nuclear chromatin and 1-2 nucleoli. The overall impression was a typical lymphocytosis and lymphoproliferative neoplasm secondary to acute lymphoblastic leukaemia. Zeng-Rong and Yufang,[49] reported that the most suspected transformations induced by chloramphenicol are acute lymphoblastic leukaemia and acute myelogenous leukaemia, both of which are derived from proliferating cells. However, graded dose of *A. muricata* was effective in modulating the lymphoblastic leukaemia effect induced by chloramphenicol in female Wistar rats as evident in Plates C-F. Onyegeme-Okerenta *et al*.[50] reported that the ethyl acetate fraction of leaf extract of *A. muricata* was found to be highly cytotoxic *in vitro* against MCF7, HT29, HCT116 and C4-2WT carcinoma cells thereby leading to non-proliferation of the cells.

Conclusion

Significant adverse changes in haematological parameters are reported to be associated with exposure to chloramphenicol in this present study. This therefore suggests that chronic intake of chloramphenicol may be considered one of the risk factors for the development of lymphoma which is secondary to leukaemia and that aqueous leaf extract of *A. muricata* have the potential to modulate the adverse effect caused by chloramphenicol administration. Hence, exposure to this drug should be under strict regulation. The potential of the aqueous leaf extract of *A. muricata* in ameliorating chloramphenicol-induced lymphoma corroborates with the reported native folkloric use of the plant in the treatment of blood-related ailments.

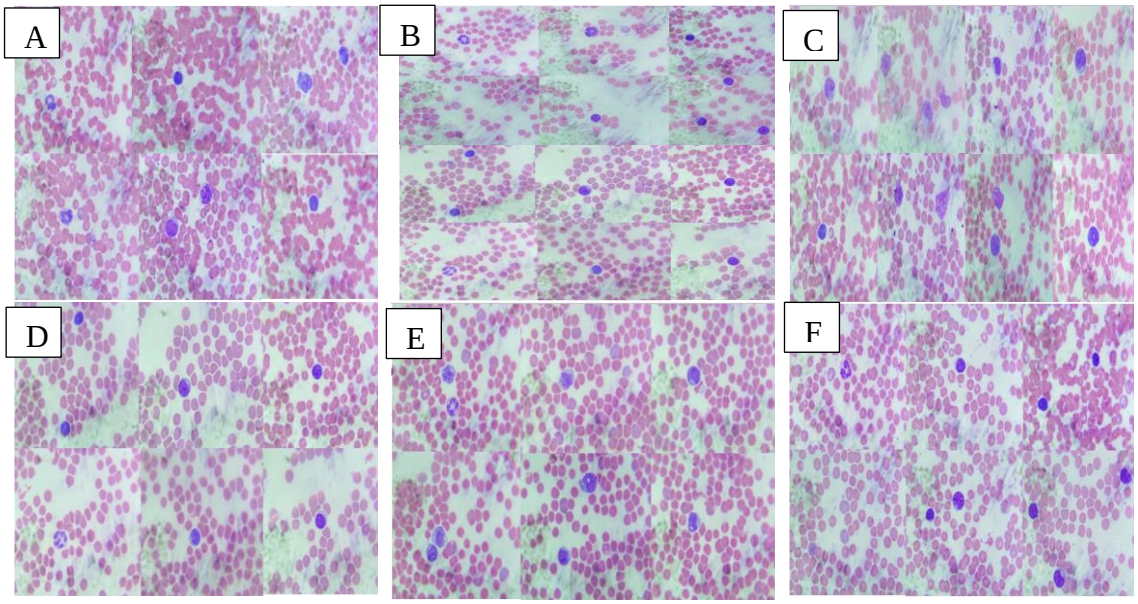

Plate 1(A – F): Photomicrographs of red blood cells from study Groups 1 to 6

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Agu KC, Okolie NP, Falodun A, Engel-Lutz N. In vitro anticancer assessments of *Annona muricata* fractions and in vitro antioxidant profile of fractions and isolated acetogenin (15-acetyl guanacone). J. Cancer Res. Pract., 2018;5(2)53-66.
2. The Global Cancer Observatory. Leukaemia. International Agency for Research on Cancer, World Health Organization, Globocan, 2020. https://gco.iarc.fr/today
3. World Cancer Report (WCR), International Agency for Research on Cancer. World Health Organization. More Than Words Inc. Waltham, Massachusetts, United States, Chapter 5.13. ISBN 978-9283204299, 2014.
4. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Le-Beau MM, Hellstrom-Lindberg E, Tefferi A, Bloomfield CD. "The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes". Blood, 2009;114(5):937–51.
5. Global Burden of Disease, (GBD). "Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015". 2015 Mortality and causes of Death, Collaborators. Lancet, 2016;388(10053):1459-1544.
6. American Cancer Society, (ACS). "Survival rates for childhood leukemia". Retrieved 17 November 2022.
7. Paul S. Kantarjian H, Jabbour EJ. Adult Acute Lymphoblastic Leukemia. Mayo Clin Proc. 2016;91:1645–1666.

8. Hutter JJ, "Childhood leukemia". Pediatr Rev., 2010;31(6):234–241.
9. Markman M, Risk factors for leukemia. Cancer treatment centers of America, https://www.cancercenter.com/cancer-types/leukemia/risk-factors, 2022.
10. Fraunfelder F, Fraunfelder F. "Restricting Topical Ocular Chloramphenicol Eye Drop Use in the United States. Did We Overreact?" Am. J. Ophthalmol., 2013;156 (3):420–422.
11. Knight M, Adverse drug reactions in neonates. J Clin Pharmacol. 1994;34(2):128-35.
12. The American Society of Health-System Pharmacists (ASHSP). Archived from the original on 2015-06-24. Retrieved December 10, 2022.
13. Drugs and Lactation Database (LactMed), Chloramphenicol. National Library of Medicine (US); Bethesda (MD), 2021.
14. Cummings ED, Kong EL, Edens MA, Gray Baby Syndrome. The United States National Library of Medicine (NLM). https://www.ncbi.nlm.nih.gov/books/NBK448133/ Retrieved 20 November, 2022.
15. Drugs.com, Chloramphenicol. https://www.drugs.com/monograph/chloramphenicol.html, Retrieved 20 November 2022.
16. Mulhall A, de Louvois J, Hurley R. "Chloramphenicol toxicity in neonates: its incidence and prevention". Br. Med. J., 1983;287(6403):1424–1427.
17. Moghadamtousi SZ, Rouhollahi E, Karimian H, Fadaeinasab M, Firoozima M, Ameen Abdulla M, Abdul Kadir H. "The chemopotential effect of *Annona muricata* leaves against azoxymethane-induced colonic aberrant crypt foci in rats and the apoptotic effect of *acetogenin annomuricin* E in HT-29 cells: a bio-assay guided approach". PLoS ONE. 2015;10(4):e0122288.
18. Deshkar SS, Mahore JG. Herbal bioactive-based vaginal and rectal drug delivery systems. In: Herbal Bioactive-Based Drug Delivery Systems: Challenges and Opportunities (1st Edition), Inderbir Singh Bakshi, Rajni Bala, Reecha Madaan, Rakesh K. Sindhu, (Editors), Academic Press, 2022;111-168. https://doi.org/10.1016/B978-0-12-824385-5.00017-0.
19. Kooti W, Servatyari K, Behzadifar M, Asadi-Samani M, Sadeghi F, Nouri B, Zare Marzouni H. Effective Medicinal Plant in Cancer Treatment, Part 2: Review Study. J Evid Based Complementary Altern Med., 2017;22(4):982–995.
20. Agu KC, Okolie NP, Eze GI, Anionye JC, Falodun A. Phytochemical analysis, toxicity profile and hemo-modulatory properties of *Annona muricata* (Soursop). Egypt J Haematol., 2017;42:36–44.
21. Onyegeme-Okerenta BM, Amadi BA, Ezeonyilimba VO. The ameliorating potential of *Annona muricata* on Sodium Fluoride-induced toxicity on liver and kidney of male wistar rats. J. complement. altern. med. res., 2018;6(1):1-17.
22. Onyegeme-Okerenta BM, Anacletus FC, Agene KR, Ubana EM. Ameliorating Potential of *Annona muricata* on Testosterone Propionate-Induced benign Prostatic Hyperplasia in Male Wistar Rats. Sch Int J Biochem, 2022;5(2):28-36.
23. Osim EE, Akpogomeh BA, Ibu JO, Eno AE. Experimental physiology manual. 3rd ed. Calabar: University of Calabar, Department of Physiology, 2004;60-81.
24. Reitman S. Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Clin. Chem., 1957;28:56-63.
25. Klein B, Read PA, Babson LA. Rapid Method for Quantitative Determination of Serum Alkaline Phosphatase. Clin. Chem. 1960:6:269-275.
26. Chuang FS, Sarbeck JR, Winefordner JD. Flame spectrometric determination of sodium, potassium and calcium in blood serum by measurement of microsamples. Clin. Chem, 2005;21:16-23.
27. Tietz NW, Clinical guide to laboratory tests 3rd Edition, W B. Saunders Co, Philadelphia, 2004;187-216.
28. Usoh IF, Akpan EJ, Etim EO, Farombi EO, Antioxidant Actions of Dried Flower extract of *Hibiscus sabdariffa* L. on Sodium arsenite-induced oxidative stress in rats. Pak J Nutr., 2005;4.3:135-141.
29. Chamcheu JC, Rady I, Chamcheu R-CN, Siddique AB, Bloch MB, Banang Mbeumi S, Babatunde AS, Uddin MB, Noubissi FK, Jurutka PW, Liu YY, Spiegelman VS, Whitfield GK, El Sayed KA. Graviola (*Annona muricata*) Exerts Anti-Proliferative, Anti-Clonogenic and Pro-Apoptotic Effects in Human Non-Melanoma Skin Cancer UW-BCC1 and A431 Cells *in Vitro*: Involvement of Hedgehog Signaling. Int. J. Mol. Sci. 2018;19(6):1791.
30. Newman DJ, Cragg GM. Natural Products as Sources of New Drugs from 1981 to 2014. J. Nat. Prod. 2016;79:629–661.
31. Tsai WC, Oith D, Van den HE. Designing memory probes to inform dialogue in proceedings of the conference on Designing interactive Systems, New York, 2017. p. 889-901.
32. Cyboran S, Oszmianski J, Kleszczyńska H. Interaction between plant polyphenols and the erythrocyte membrane. Cell Mol Biol Lett. 2012;17(1):77-88.
33. Onyegeme-Okerenta BM, Omeje HC, Ogunka-Nnoka CU. Biochemical evaluation of chloramphenicol-induced lymphoma and ameliorative potentials of *Justicia c*arnea and *Cnidoscolus aconitifolius* in male Wistar rats. GSC Adv. Res. Rev., 2021;09(01):128–136.
34. Lala V, Zubair M, Minter DA. Liver Function Tests. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482489/.
35. Paya CV, Wiesner RH, Hermans PE, Larson-Keller JJ, Krom RA, Smith TF. Risk factors for cytomegalovirus and severe bacterial infections following liver implantation: a prospective multivariate time-dependent analysis. J. Hepatol., 1993;18(2): 185-95.
36. Dou L, Shi M, Song J, Niu X, Niu J, Wei S, Li D, Bai Y, Sun K. The Prognostic Significance of C - reactive protein to Albumin Ratio in Newly Diagnosed Acute Myeloid Leukaemia Patients. Cancer Manag Res. 2022;14:303-316.
37. Lucijanic M, Galusic D, Krecak I, Sedinic M, Soric E, Holik H, Perisa V, Moric Peric M, Zekanovic I, Stoos-Veic T, Kusec R. C reactive protein to albumin ratio as prognostic marker in primary and secondary myelofibrosis. Leuk Lymphoma. 2020;61(12):2969-2974.
38. Nozoe T, Matono R, Ijichi H, Ohga T, Ezaki T. Glasgow prognostic score (GPS) can be a useful indicator to determine prognosis of patients with colorectal carcinoma. Int Surg. 2014;99(5):512–7.
39. Herishanu Y, Polliack A, Shenhar-Tsarfaty S, Weinberger R, Gelman R, Ziv-Baran T, Zeltser D, Shapira I, Berliner S, Rogowski O. Increased serum C-reactive protein levels are associated with shorter survival and development of second cancers in chronic lymphocytic leukaemia. Ann. Med. 2017;49(1):75-82.
40. Simoes R, Silva LM, Cruz ALVM, Fraga VG, de-Paula Sabino A, Gomes KB. Troponin as a cardiotoxicity marker in breast cancer patients receiving anthracycline-based chemotherapy: A narrative review. Biomed. pharmacother., 2018;107:989-996.
41. Zafar Gondal A, Foris LA, Richards JR. Serum Myoglobin. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470441/
42. Bicker A, Nauth T, Gerst D, Aboouf MA, Fandrey J, Kristiansen G, Gorr TA, Hankeln T. The role of myoglobin in epithelial cancers: Insights from transcriptomics. Int J Mol Med. 2020;45(2):385-400.
43. Felsenfeld AJ, Levine BS, Rodriguez M. Pathophysiology of calcium, phosphorus, and magnesium dysregulation in chronic kidney disease. Semin Dial. 2015;28:564–577.
44. George VC, Kumar DR, Rajkumar V, Suresh PK, Ashok K. Quantitative assessment of the relative antineoplastic

potential of the n-butanolic leaf extract of *Annona muricata* Linn. In normal and immortalized human cell lines. Asian Pac. J. Cancer Prev., 2012;13:699-704.
45. Nawwar M, Ayoub N, Hussein S, Hashim A, El-Sharawy R, Wende K, Harms M, Lindequist U. A flavonol Triglycoside and investigation of the antioxidante and cell stimulating activities of *Annona muricata* Linn. Arch. Pharmacal Res., 35 (2012) 761-767.
46. Alitonou GA, Tchobo FP, Sessou P, Avlessi F, Menut C, Sohounhloue DCK. Chemical composition, antiradical and anti-inflammatory activities of four *annonaceae* from Benin. Int. J. Pharm. Chem. Biol. Sci., 2013;3:914-923.
47. Vit P, Santiago B, Perez-Perez ME. Chemical composition and antioxidant activity of the pulp, leaves and seeds of soursop *Annona muricata* L Interciencia, 2014;39(5):350-353.
48. Coria-Tellez AV, Montalvo-Gonzalez E, Yahia EM, Obledo-Vazquez EN. *Annona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity, Arab. J. Chem, 2018;11(5):662-691.
49. Zeng-Rong Y, Yufang S. Chloramphenicol induces abnormal differentiation and inhibits apoptosis in activated T cells. Cancer Res., 2008;68(12):4875-4881.
50. Onyegeme-Okerenta BM, Bradshaw T, Spriggs K. Anti-proliferative Activity of Ethyl Acetate Leaf Extract of *Annona Muricata* L. on Selected Carcinoma Human Cell Lines. LJRS: Natural and Formal, 2018; 18(4):19-34.