

Therapeutic Potentials of *Acacia Nilotica* Aqueous Extract in Benzene -Induced Leukemia: Insights into Hepatic and Renal Protection

Christiana Joseph Bala ¹; Samiu Olalakan Ajiboso ¹; Ann Ukamaka Ijeomah ¹;
Kebe Etim Arikpo ¹; Moses Zira Zaruwa ¹; and Ukoma Chi-kadibia Theophilus ²

¹Biochemistry Department, Nasarawa State University, Keffi, Nigeria. ²Haematology Department, Federal Medical Center, Keffi, Nigeria.

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Abstract

Leukemia is a malignancy arising as a result of mutations in immature white blood cells called blasts that leads to growth and proliferation arrest during hematopoiesis. Medicinal plants have the ancient history of human use and have been considered one of the important and reliable sources to discover promising therapeutic agents in disease conditions, including leukemia. *Acacia nilotica* can provide the nutrients and therapeutic ingredients to prevent, mitigate or treat many diseases due to the presence of bioactive compounds. Phytochemical screening revealed the presence of alkaloids, tannins, and phytosterol in *Acacia nilotica*. Leukemia was induced in albino rats using benzene solution. Different concentrations (7.5, 10, and 12.5 mg) of *Acacia nilotica* extract were administered to leukemic rats. Hematological parameters, liver enzymes, lactate dehydrogenase (LDH), antioxidants, electrolytes, C-reactive protein (CRP), and uric acid levels were determined and compared with control groups consisting of a negative control, positive control and chemotherapeutic standard drug of doxorubicin and cyterabin control groups. *Acacia nilotica* produced higher WBC values ($16.95 \times 10^9/L$ to $14.00 \times 10^9/L$), which decreased with increasing dose but remained above control levels, likely due to non-malignant stimulation and its high phytosterol content. Red blood cell (RBC) counts were $7.95 \times 10^{12}/L$, $6.69 \times 10^{12}/L$, and $6.46 \times 10^{12}/L$ for negative, positive, and standard controls, respectively. LDH results were 367 U/L, 1844 U/L, and 377 U/L for negative, positive, and standard controls, respectively. *Acacia nilotica* 758 U/L at 7.5 mg. Sodium ion concentrations were 131 mmol/L, 111.4 mmol/L, and 132 mmol/L for negative, positive, and standard controls, respectively. Other electrolytes, CRP, and uric acid levels remained significantly ($P < 0.05$) within normal ranges after treatment. The effects of the extracts were comparable to those of the standard drug (Doxorubicin/AraC), demonstrating similar therapeutic properties. Findings from this research validate the traditional and chemotherapeutic use of *Acacia nilotica* in the management of leukemia due to the presence of bioactive compounds like alkaloids, antioxidant capability and immunomodulatory role. Further studies should focus on isolation of bioactive constituents to develop standard phyto-pharmaceuticals and elucidating their mechanism of action.

Keywords: Benzene, Leukemia, *Acacia nilotica*, antioxidant, Anti-leukemic, Gum Arabic and Immunomodulation.

Introduction

Cancer is one of the leading causes of death in the world (Rajabli, 2013; Izuegbuna, 2021). It was estimated that 12 million new cases of cancer occurred all over the world and 7.6 million deaths (13% of all deaths) in 2024 (GLOBACAN 2024). These numbers are expected to double by 2030, the age-standardized incidence rates (ASR) of all cancers were 202.8 and 164.4 per 100,000 person-yearly among male and female, respectively (Rajabli *et al.*, 2013; Izuegbuna, 2021). Cancer was once considered a problem exclusive to western countries, but it is becoming a leading cause of death and disability in low- and middle-income countries (LMICs) such as Asia, Africa and part of South America (Shailja *et al.*, 2019; Izuegbuna, 2021). Leukemia a type of cancer has been proven to be a heterogeneous group of hematopoietic malignancy (Rajabli *et al.*, 2013). They are group of malignant disorder in blood and blood-forming tissues it is a common malignancy that present itself with increased numbers of leucocytes (White Blood Cells, WBCs) (Arikpo *et al.*, 2025). Leukemia occurs as a result of mutations due to radiation or exposure to carcinogenic substances such as benzene in immature white blood cells called blasts that leads to growth arrest and subsequent proliferation of the blasts in the bone marrow, the blood-forming tissues of the body, causing the overproduction of these immature blood cells that enter the blood stream (Sanal *et al.*, 2015; Olufemi *et al.*, 2017; Kabeel *et al.*, 2018; Maher *et al.*, 2021 Liu *et al.*, 2022). In 2018, leukemia accounted for more than 430,000 new cases of all cancers worldwide; with lymphomas and myeloma, the number exceeded more than 1.1 million new cases (Izuegbuna, 2021). According to Rajabli *et al.*, 2013; Tayebah *et al.*, 2014; Sanal *et al.*, 2015, there are four major types of leukemia which are; Acute Myeloid Leukemia (AML), Acute Lymphoid Leukemia (ALL), Chronic Myeloid Leukemia (CML) and Chronic Lymphoid Leukemia (CLL). Industrial exposure to benzene, cigarette smoking, emissions of gasoline vapour and petroleum products at the filling stations, in cars and in garages attached to homes have become major concern regarding human health in the 21st century as living standards in many countries around the world has risen and more people drive gasoline-fueled cars. Emissions from motorized traffic, which include benzene, have been hypothesized to increase the risk of childhood leukemia, based on results from recent epidemiologic and toxicological studies. The number of people who are exposed to benzene has expanded dramatically (Snyder, 2012; Vinceti *et al.*, 2012). Benzene depresses white blood cell count (leukopenia) on prolonged exposure and should not be used as a laboratory solvent (Kareem, 2022). Activation of benzene and its reactive metabolites leads to continuous production of reactive oxygen species (ROS), which damages DNA, RNA, and proteins by chemical reactions such as oxidation, nitration, and halogenations leading to genetic modification and alterations in the functions of important enzymes and proteins (Uzma *et al.*, 2010).

Medicinal plants have the ancient history of human use and have been considered one of the important and reliable sources to discover promising therapeutic agents in disease conditions, including leukemia drugs (Tayebah *et al.*, 2014). Medicinal plants have a long history of being used to cure numerous illnesses and different forms of cancer (Maher *et al.*,

2021). It is estimated by World Health Organization that around 80% of Africans are currently using traditional medicines to manage their prevailing ailments (Abdelgawad *et al.*, 2023). There has been report of improvement in the treatment of cancer using secondary compounds from natural products and medicinal herbs. (Kooti *et al.*, 2017). Plant-based bio-active compounds are considered as renewable sources of anti-leukemic agents due to their diversity and availability (Arikpo *et al.*, 2025). As of 2013, 1,453 new chemical entities (NCEs) have been approved by the US FDA, of which some are natural products or natural product-inspired. Natural Compounds such as the vinca alkaloids listed below function in the treatment of cancer as microtubule inhibitors. They do this by inhibiting the polymerization of tubulin which is necessary in the formation of spindle at the M-phase of cell cycle. At low doses they disrupt microtubular function but at high doses cell cycle arrest and apoptosis occur (Chen and Menezes, 2024).

Acacia nilotica (A.N) also called gum Arabic is a perennial tree found growing wild in large commercial quantity in the Northern parts of Nigeria (Adindu *et al.*, 2019). It is indigenous to Africa and Asia. Exudate of *Acacia nilotica* and related tree species, are essential ingredient of many industrial products such as soft drinks, candies, also used as a thickener, emulsifier, food preparation and in other industries like cosmetics and pharmaceuticals (USAID AFRiSD, 2001; Akram, 2020). *Acacia nilotica* is also known as acacia fiber because of its high fiber content, eating high fiber is good for gut, weight reduction, protect against diabetes, and ease irritable bowel syndrome symptoms and heart (USAID AFRiSD, 2001). *Acacia nilotica* exhibit some valuable properties, for instance, emulsification, adjustment, and microencapsulation (Akram, 2020). *Acacia nilotica* is a good source of natural antioxidants (Akram, 2020). It helps the digestion of lipids. It is used against a few sicknesses, like renal failure, cardiovascular, and gastrointestinal diseases (Akram, 2020). *Acacia nilotica* has cancer prevention properties, due to its coordinated radical scavenging capacity (Akram, 2020). There is growing confirmation that the antioxidant capacity is due to its protein fraction, in a general sense by amino acids, which are generally considered as cancer prevention agents (Akram, 2020). *A. nilotica* also has anti-plasmodial, molluscidal, anti-fungal, anti-microbial activity, inhibitory activity against HCV and HIV-I (Ali *et al.*, 2012). The bark of the plant is used as astringent, acrid, cooling, styptic, emollient, anthelmintic, etc. It is used by traditional healers of different regions of Chattisgarh in treatment of various cancer types of mouth, bone and skin (Ali *et al.*, 2012). In West Africa, the bark and gum are used against cancers and/or tumors (of ear, eye, or testicles) and indurations of liver and spleen, the root for tuberculosis, the wood for smallpox and the leaves for ulcers (Ali *et al.*, 2012).

Presently, various methods are used for leukemia treatment such as chemotherapy, radiation, surgery etc, but because of non-selectivity of some of these therapeutic drugs and their adverse effects, a high percentage of healthy cells are destroyed alongside with cancer cells. Many factors are related to the development of leukaemia and orthodox treatment such as chemotherapy and ionizing radiation has been found to be one of the factors that predisposes patients to leukemia (Tayebeh *et al.*, 2014; Adewale *et al.*, 2020).

The issue of orthodox drug resistance such as resistance of 1st and 2nd generation tyrosine kinase inhibitors (TKI) by chronic myeloid leukemia has also made leukemia treatment a challenge (Izuegbuna, 2021). The most challenging problem in cancer treatment is the selective destruction of tumor cells without affecting the normal cells. It is therefore important to discover new active bio-active components against cancer (leukemia) (Kooti *et al.*, 2017). The need for less expensive alternative strategies such as traditional plants and herbs to increase treatment access and survival outcomes for all patients comes necessary because of the limitations of the existing drugs (Allqmani *et al.*, 2020). Therefore, availability of natural products with higher effectiveness and lower side effects is desired (Kooti *et al.*, 2017). Recent advances in alternative and complementary medicine have shown that various herbs, fruits, and vegetables contain compounds that could act as inhibitors of cancer formation, blockers of carcinogen interaction, and suppressors of tumor progression (Allqmani *et al.*, 2020). Therefore, this study was designed to evaluate the phytochemistry, hepato and nephro- protection, antioxidant and anti-leukemic potentials of *Acacia nilotica* in benzene induced leukemic rats

Research Methodology

Chemicals

All reagents that were used for this research were of analytical grade. The reagent include; Ethanol, Bovine Serum Albumin (Sigma), Benzene, Propanol, Doxorubicin and Ara-A (orthodox drugs used for treating leukemia).

Plant Material

Acacia nilotica was bought in Keffi town market in Nasarawa State, Nigeria. All the parts gotten were thoroughly washed and identified using Virtual Botanic Garden (VIRBOGA) Dataset Identifier in Plant Science Department NSUK, Nasarawa State. They were all air dried under laboratory conditions for two weeks in the Department of Biochemistry, Nasarawa State University, Keffi (Arikpo *et al.*, 2025).

Equipment's

The equipments used for this research includes; Spectrophotometer (JENWAY 7305), Hotplate, Soxhlet Apparatus, Rotary Evaporator (RE300), Trinocular Microscope (Swift SW350T 40X-2500X) and Microplate Reader (NeoMedica NW-67).

Experimental Animals

Thirty (30) male Albino rats were used for the study. The rats were obtained from the Animal house in Vom, Jos Plateau State weighing between 190-220g. The rats were randomly arranged in twelve compartment zinc cages, with n = 5 per group. They were allowed to acclimatize to laboratory condition for two weeks before the beginning of the experiments according to These animals were maintained at room temperature with a 12h

light/12h dark cycle and were allowed *ad libitum* access to feed and water (Arikpo *et al.*, 2025).

Ethical Consideration

Ethical approval was obtained from NSUK Animal Care and Use Research Ethics Committee (NSUK-ACUREC). All experimental steps were made according to the ethical rules of NSUK-ACUREC, Nasarawa State University, Keffi.

Extraction of the Plant Sample

The dried plants were grinded to pass a 0.5 mm sieve which provided a greater surface area, they were weighed thereafter all according to Arikpo *et al.*, 2025. An average of 14g of the dried powder were filled in whatman filter paper 1 and subjected to soxhlet extraction using deionized water for 12 hours at 65°C. The aqueous extracts obtained were concentrated to dryness at 45°C using a rotary evaporator under reduced pressure and the extract were weighed and then stored at 4°C for further use (Arikpo *et al.*, 2025).

Phytochemical Analysis

Qualitative Phytochemical Screening of the Extracts

The qualitative phytochemical screening was done according to the method by Vishnu *et al.* (2019) in a laboratory at Sheda Science and Technology Complex (SHESTCO). Comprehensive description of the procedure is recorded at the Appendices.

Induction of Leukemia

Benzene solution, a product of Sigma-Aldrich with Cat No 270709 and >99.9% was diluted in water and propanol which was used for the induction of leukemia. The composition of the injection is 1 ml of the benzene, 4mls of propanol and 5mls of water (1:4:5). Leukemia was then induced into Wister rats by intravenous injection of 0.2 mLs of the 1:4:5 diluted benzene solution (Benzene, propanol, water), the induction was carried out every 2 days (48hourly) for 3 consecutive weeks according to Kebe *et al.*, 2025 with modification. After induction, hematological (WBC with the differentials; Neutrophils, monocytes, lymphocytes, eosinophils and basophils; RBC, HGB and Platelets) properties, liver (AST, ALP and ALT) enzymes, LDH, C-Reactive protein, Uric acids, electrolytes, creatinine and histology of all blood samples from the rats were analyzed. The results from these induced rats were compared with those of leukemic rats from literature which showed that these rats have become leukemic. The following were observed to further confirm the presence of leukemia; weight loss, weakness of the body seen by the activities of the rats, some had paralysis of the hind legs and some died.

Experimental Design

GROUPS	TREATMENT
Groups 1: negative control	Feed and water only
Group 2: positive control	no treatment; Benzene without treatment
Group 3: (Dox/Ara C)	was treated with doxorubicin (0.6mg) in combination of Ara-C (0.2mg) in a 3 to 5 days regiments (orthodox medicine) respectively.
Group 4	was treated with 7.5mg/10mls water extract of <i>Acacia nilotica</i>
Group 5	was treated with 10mg/10mls water extract of <i>Acacia nilotica</i>
Group 6	was treated with 12.5mg/10mls of water extract of <i>Acacia nilotica</i>

Animal Sacrifice

After 3 weeks of benzene injection and 3 weeks of administration of plant aqueous extracts (as designed in the experimental protocol above), animals in the respective groups were sacrificed. Blood was collected into ethylenediaminetetraacetic acid (EDTA) vials and heparin vials, gently mixed, labeled, and analyzed. The animals were handled and sacrificed ethically as recommended by animal care regulations.

Blood Samples Analysis

Haematological Analysis

The haematological parameters of the blood of the animals such as hematocrit, total white blood cell (WBC) counts with defferentials, red blood cell (RBC) counts, hemo-globin (Hb) and platelet count estimation using the automated blood analyzer (SYSMEX KX21) were analyzed in all the twelve (12) groups;

Principle

A micro-pore was placed in the blood to be measured and electrodes of a certain voltage were added at the end of the micro-pore. When the particles in the liquid pass through the micro-pore, the resistance between the electrodes produced an instantaneous change. As a result, an electrical pulse was generated. The number of particles generated were counted by the electrical pulse. The size of the pulse amplitude indicates the size of the volume. The different types of cells are distinguished by selecting the electrons of the size of the pulses generated by each cell.

(Coulter, 2022).

1. Blood cells are suspended in a conductive liquid
2. The cell suspension passes through a small aperture
3. The electrical current changes as the cells pass through the aperture
4. The changed in electrical current are measured to count and size the cells.

Determination of Liver Enzymes

Principles and Method for Alkaline Phosphatase (ALP) Assay

Principles: Serum or Plasma alkaline phosphatase hydrolyzes a colourless substrate of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein which, at alkaline pH values, turns into a pink colour that can be photometrically determined (Odiegwu *et al.*, 2021).

Principles and Method for Alanine Transaminase (ALT) Assay

Principle

ALT catalyses the reversible transamination of L-alanine and α -ketoglutarate into pyruvate and L-glutamate. The pyruvate is then converted to lactate-by-lactate dehydrogenase and NADH, which is then oxidized to NAD⁺. The rate of this reaction, measured by the decrease in NADH absorbance at 340 nm, is directly proportional to the ALT activity in the sample.

Method

Sample were transferred into Tecan tubes (Sarstedt, Germany). The transferred plasma was analyzed on ILab Taurus instrumentation (Werfen, Spain), and then frozen at – 20 °C for subsequent analyses. AST and ALT values on the ILab Taurus were obtained using the *traditional* manufacturer's reagent and the new reagent formulation (from the same manufacturer) that include Pyridoxal-5'-Phosphate (PLP) as an additional reagent which reaches a concentration of 0.1 mmol/L in the reaction (Talli *et al.*, 2025).

Principles and Method for Aspartate Transaminase (AST) Assay

Principles

AST in the sample catalyses the transfer of an amino group between Aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH) to form NAD⁺. The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance (Roche Diagnostic, 2011).

Determination of Lactate Dehydrogenase Enzyme

LDH Assay Kit (Cytotoxicity) ab65393 uses Water Soluble Tetrazolium salt (WST) for the fast and sensitive detection of LDH released from damaged cells. The LDH assay protocol was based on an enzymatic coupling reaction: LDH released from the cell oxidizes lactate to generate NADH, which then reacts with WST and generated a yellow color. The intensity of the generated color correlates directly with the number of lysed cells.

Principle and Method

The Seralyzer lactate dehydrogenase method is based on the conversion of pyruvate to lactate, at a temperature of 37°C and a total reaction time of 120 s. A measured amount of serum-diluted threefold-is added to the strip, which contains pyruvate and NADH. Lactate

dehydrogenase activity is measured by means of reflectance spectroscopy, by the rate of disappearance of NADH at 340 nm with reference to a calibration curve generated using lactate dehydrogenase calibrators (Newall, 1984).

Antioxidant Determination

Determination of Superoxide Dismutase (SOD) Activity

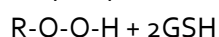
Superoxide dismutase (SOD) was determined using Cayman's Superoxide Dismutase Assay Kit (No: 706002). Cayman's Superoxide Dismutase Assay Kit utilises a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine.

Determination of Catalase (CAT) Activity

The catalase activity was determined using Cayman Catalase Assay Kit (No: 707002). Cayman Catalase Assay Kit utilizes the peroxidatic function of CAT for determination of enzyme activity. This method was based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2 (Havel, 2019). The formaldehyde that was produced was measured colorimetrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (purpald) as the chromogen. Purpald specifically form a bicyclic heterocyclic with aldehydes, which upon oxidation changes from colourless to a purple colour. Detailed procedures stated in Appendices.

Determination of Glutathione Peroxidase (GPx) Activity

Glutathione Peroxidase (GPx) was determined using Cayman Glutathione Peroxidase Assay Kit (No: 703102). Cayman GPx assay measures GPx activity indirectly by a couple of reaction with Glutathione Reductase (GR). Oxidised glutathione (GSSG), produced upon reduction of hydroperoxide by GPx was recycled to its reduced state by GR and NADPH.



The oxidation of NADPH to $NADP^+$ was accompanied by a decrease in the absorbance at 340 nm. Under condition in which the GPx is rate limiting, the rate of decrease in the A_{340} was directly proportional to the GPx activity in the sample. Procedure in Appendices.

Determination of Reduced Glutathione (GSH)

The glutathione (GSH) was determined using Cayman Glutathione Assay Kit (703002). Cayman glutathione assay utilizes a carefully optimizing enzymatic recycling method glutathione reductase (GR) for the quantification of GSH. The sulfhydryl group of GSH reacts with DTNB [5,5'-dithio-bis-2-(dinitrobenzoic acid), Ellman's reagent) and produces a yellow coloured 5-thio-2-nitrobenzoic acid (TNB). The mixed disulphide, GSTNB (between GSH and TNB) that is concomitantly produced is reduced by GR to recycle GSH and produce more TNB. The rate of TNB is directly proportional this recycling reaction which is in turn directly proportional to concentration of GSH in the sample. Measurement of the absorbance of TNB at 405-414 nm provide an accurate estimation of GSH in the sample. Procedure in Appendices.

Determination of Lipid Peroxidation

Lipid Peroxidation was determined by measuring the Thiobarbituric Acid Reactive Substance (TBARS) using Cayman TBARS Assay Kit. Cayman TBARS Assay Kit provide a simple, reproducible and standardized tool for assaying lipid peroxidation in plasma, serum, urine, tissues homogenates and cell lysates. The MDA-TBA adduct that was formed by the reaction of Malondialdehyde (MDA) and Thiobarbituric Acid (TBA) under high temperature (90-100°C) and acid condition was measured colorimetrically at 530-540 nm. Procedure in Appendices.

Determination of Electrolytes

K⁺, Na⁺, and Cl⁻ were estimated by direct kit method using precipitants of Spinreact Co, Spain (Rasool *et al.*, 2014).

Determination of Urea

Urease (10mg, specific activity of 1500 U/mg protein) was dissolved in 10 ml of 25mM tris-acetate buffer, pH 5.5. To this solution 5 mg of phenol red dye was added and mixed thoroughly to make a homogeneous solution. The stability of the enzyme was increased by adding 100 µl β-mercaptoethanol and centrifuged at 5000 rpm for 5 min to obtain a clear enzyme solution. Whatman No. 1 paper (5x45cm) was coated with enzyme dye solution and dried at 30°C in humidity free chamber. The color of the paper changes to light yellow after complete drying. The paper was cut (5 m. width) into several pieces.

High impact polymer plastic sheet (1 mm. Thickness) was cut into the size of 9x90 cm. The coated piece of paper was pasted to the plastic sheet towards the length using suitable non-reactive adhesive (PSV synthetic resin adhesive diluted, 1:10 w/v with water). After complete drying, the plastic sheet, attached with paper, was cut into the size of 0.5x9.0 cm. Thus, one end of the strip is having reactive enzymatic pad and the other one is used for handling the strip.

In-vivo Determination of Creatinine

Creatinine

A 10-µl aliquot of serum sample was diluted with 740 µl of distilled water and fortified with 500 µl of 1 µg/ml creatinine-D₃ solution (C/D/N Isotopes Inc., Quebec, Canada). Three volumes of methanol were added to the mixture to precipitate out proteins. After vortexing for 1 min and centrifugation at 12,000g for 10 min at 4°C, 500 µl of the supernatant was collected for further analysis. The quantitative LC-MS/MS analysis of creatinine was performed on an Agilent 1200 series SL RRLC system coupled to an Agilent 6410 triple quadrupole mass spectrometer equipped with an ESI source in positive mode (Agilent Tech., Santa Clara, CA). One microliter of the sample was injected onto a 2.1 mm × 100 mm Kinetex 2.6-µm HILIC column (Phenomenex Inc., Torrance, CA) with a mobile phase consisting of 0.1% formic acid in water (A) and in acetonitrile (B) at a constant flow rate of 0.3 ml/min. The following linear gradient program for elution was applied: 0–1 min on 90%

B, 1–5 min linearly which decreased from 90% to 40% B, and was held for 1 min before returning to initial condition. Optimized MS parameters was follows as: capillary voltage of 4,000 V, drying gas temperature of 350°C, drying gas flow rate of 8 l/min, nitrogen nebulizer pressure of 30 psi, and dwell time was 200 msec. The detection was carried out by the multiple reaction monitoring (MRM) modes. The fragmentor voltage (V), collision energy (V), and MRM transitions monitored followed: 102, 15, m/z 117→47 for creatinine-D₃; 100, 20, m/z 114→44 for creatinine.

***In vivo* C-Reactive Protein Analysis in Leukemic Rats**

The anti-inflammatory activity occurring in the benzene induced rats was measured using C-reactive protein analysis (Precision Biomed Pvt.Ltd, 2021). The animals were divided as in the experimental design:

The serum of all the samples and reagents were brought to room temperature and gently mixed with latex reagent prior to use. One drop (40 µl) of serum from both positive and negative control were dropped on a glass slide. Then 1 drop of CRP latex reagent was added to all the slides.

The separate mixing sticks were mixed and the fluid spread over the entire area of the cell. The slide back was tilted forth slowly for 2 minutes observing preferably under artificial light. Then agglutination was observed.

***In-vivo* Determination of Uric Acid Concentration**

Uric acid concentration was determined according to the method of Trinder as contained in Biotrust assay kit, 1969. Uric acid is oxidized to allantoin and hydrogen peroxide in the presence of uricase. The liberated hydrogen peroxide was detected by a chromogenic oxygen acceptor in the presence of peroxidase. The red quinone formed was proportional to the amount of uric acid present in the sample.

Histopathology Examination

Peripheral blood smear using kir-ral staining kit was used to examine and compare the presence and number of red, white blood cells and platelets under electron microscope. This was done to observe the differences on the different slides prepared from the different samples taken from different experimental groups and different control groups.

Statistical Analysis

All data obtained were analyzed using SPSS statistical software version 21. For comparison of multiple groups, one-way analysis of variance (ANOVA), with a Turkey's post hoc test for homogeneous subset were used to define statistically significant differences among the groups. Probability values ($P < 0.05$, $p < 0.01$ and $p < 0.001$) were considered as statistically significant.

Results

Table 1: Qualitative Phytochemical Constituents of *Acacia nilotica* extract

The table below is the result of the phytochemical constituents in *Acacia nilotica*, alkaloids, tannins, cholesterol and phytosterol were present in cupious amounts

Phytochemicals in <i>Acacia nilotica</i>	
Flavonoids	-
Alkaloids	+
Quinones	-
Phenols	-
Triterpenoids	-
Reducing sugars	-
Tanins	+
phytosterol	+
Anthocyanins	-
Resins	-
Saponins	-

+ = present, - = absent

Plate 1: Effects of *Acacia nilotica* extracts on peripheral blood examination

Plate **1A** shows histology of negative control where all the blood cells are in normal proportion, **1B** shows infiltration of blast cells replacing normal hematopoietic cells and **1C** shows histology of blood from the group treated with doxorubicin/Ara-C where both healthy and non-healthy cells are reduced. The smears **1D, E** and **G** is *Acacia nilotica* extracts treated groups of 7.5mg/10mls H₂O, 10.0mg/10mls H₂O and 12.5mg/10mls H₂O shows WBC, RBC and Platelets morphology with reduced leukemic infiltration (on a dose dependent manner) compared to the positive control (**1b**).

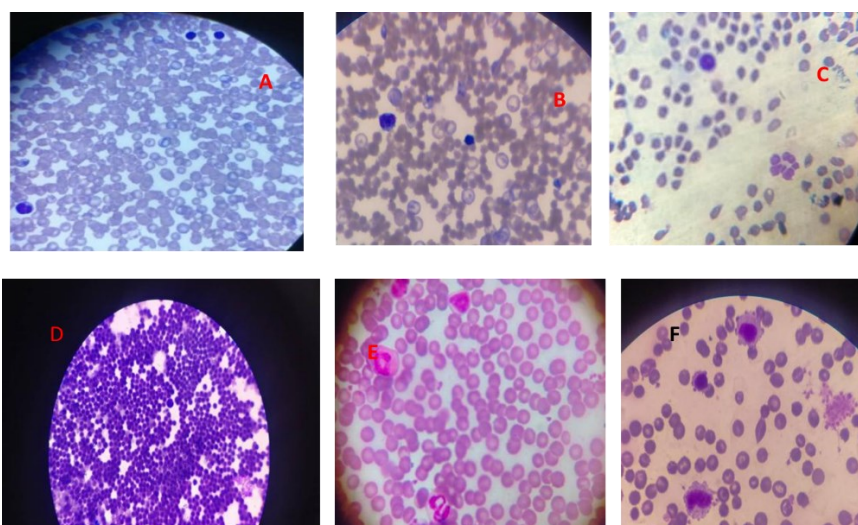
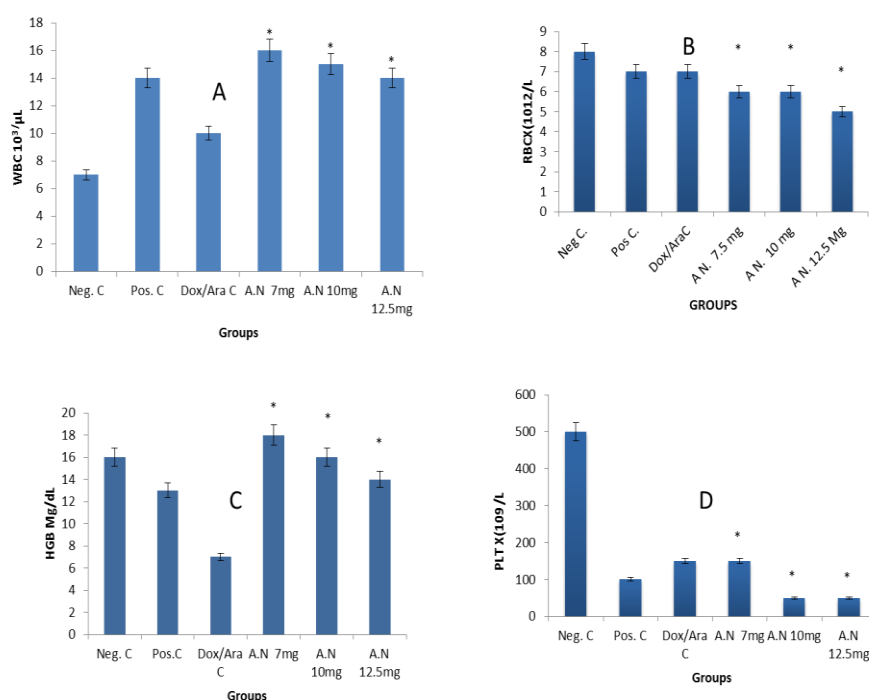


Plate 1 A= negative control, B= Positive control, C= Dox/ Ara C, D, E and F are 7, 10 and 12.5 mg of *Acacia nilotica* extract

Effect of *Acacia nilotica* extracts on White Blood Cells (WBC), Red Blood Cells (RBC), Haemoglobin (Hb) and Platelets (PLT) in Benzene Induce Leukemic wistar rats

Figure 1 showed WBC level to be higher in positive control compared to the negative control. The level in standard control is lower than that of positive control but in the same range with that of negative control. All the groups treated with 7.5 mg, 10 mg and 12.5 mg *Acacia nilotica* are significantly ($p<0.05$) higher WBC compared to the control groups which may be due to effects of the extracts. Figure 1 also depicts the level of RBC's which are lower in positive control compared to the level in negative control. RBC were significantly ($p<0.05$) low in all the group treated 12.5 mg gum Arabic extracts compared to the negative and standard controls. The groups treated with 7.5 and 10 mg gum Arabic were slightly higher than the standard and negative control groups. Hemoglobin's concentration is lower in the positive control compared to the concentration in the negative control. The concentration of hemoglobin where significantly ($p<0.05$) higher in all the *Acacia nilotica* treated groups (7.5, 10 and 12.5 mg) compared to the standard control group (figure 1). Platelets counts was significantly ($p<0.001$) low in the positive control compared to the negative control and standard control is higher than the positive control. All the treatment groups including the standard drug were significantly ($p<0.001$) low, compared to the negative control. A.N and the standard drug had different effects on platelets but generally causing thrombocytopenia.

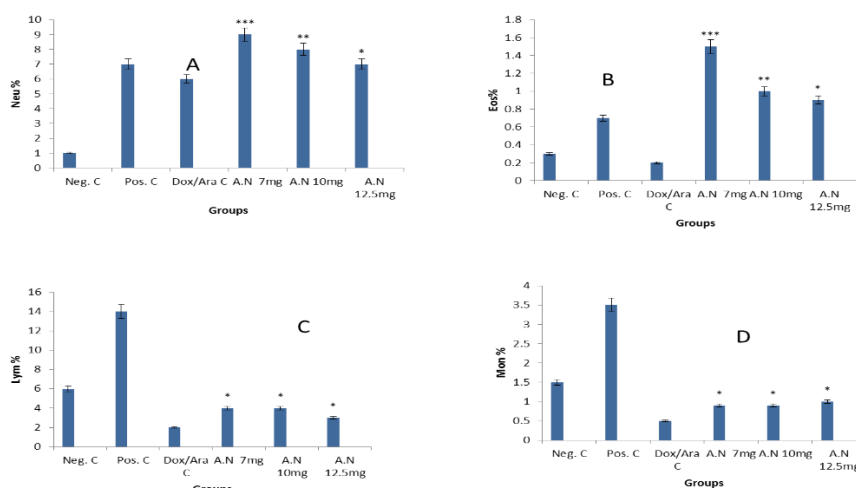


Results represented as mean $\pm$ SD (n=5). Symbols represent the level of significance *= $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

Fig.1 Effect of *Acacia nilotica* extract on White blood cells (A), Red blood cells (B), Haemoglobin (C) and Platelets(D).

Effect of *Acacia nilotica* extracts on Neutrophils (Neu), Eosinophils (Eos), lymphocyte (Lym) and Monocytes (Mon) in Benzene Induce Leukemic wistar rats

As seen in figure 2A, neutrophils were found to be significantly ($p < 0.05$) very high in the positive control group compared to the negative control group. Groups treated with 7.5, 10 and 12.5 mg *Acacia nilotica* had neutrophils significantly ($p < 0.05$) higher compared to the standard control group. The high level of neutrophils in the groups treated with *Acacia nilotica* maybe due to the machine picking and counting all blasts as neutrophils since the nucleus at early stage looks same. Other factors responsible for the high level may be tissue damage and inflammation due to the disease. Figure 2B shows a significantly ($p < 0.05$) high amounts of eosinophils in the groups treated with *Acacia nilotica* extracts compared to the standard control. Benzene metabolism which led to the induction of leukemia, chronic inflammation and oxidative stress/immune deregulation (which are all associated with leukemia) might all have contributed to the different levels of eosinophils in different plant groups. Figure 2C shows a significantly ($p < 0.05$) high amounts of lymphocytes in the positive control compared to the negative control. The amount in standard control is significantly ($p < 0.01$) low compared to the positive control due to cytotoxicity. All the groups treated with *Acacia nilotica* extracts were significantly ($p < 0.05$) lower compared to positive control group, with different varying degrees. Figure 2D shows a significantly ($p < 0.001$) high amounts of monocyte in the positive control compared with the negative control. The standard control is significantly ($p < 0.05$) low compared to the positive control. The groups treated with *Acacia nilotica* extracts (7.5, 10, 12.5 mg) were significantly ($p < 0.05$) higher compared to the standard control group. All the treatment group showed therapeutic properties

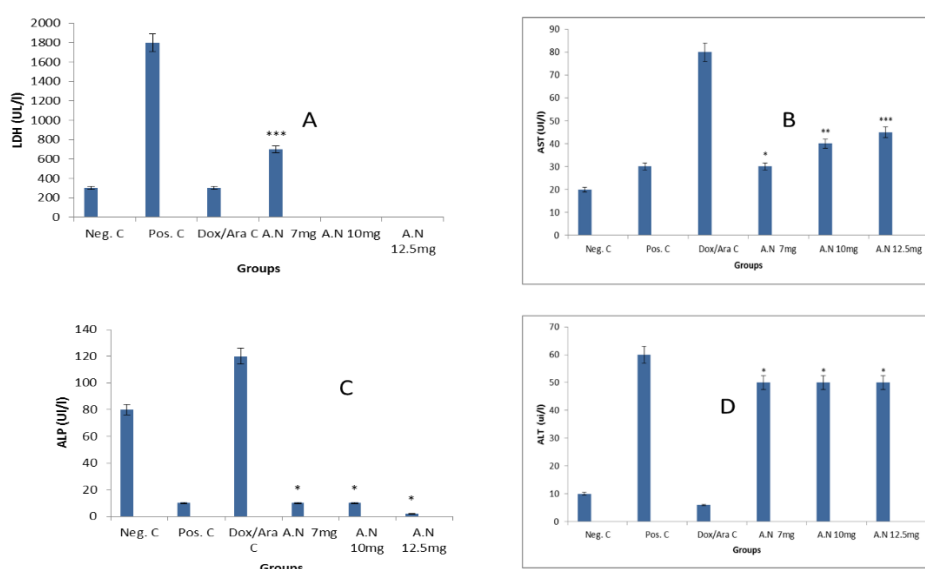


Results represented as mean $\pm$ SD ($n=5$). Symbols represent the level of significance *= $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

Fig. 2 Effect of *Acacia nilotica* extracts on Neutrophils (A), Eosinophils (B), lymphocyte (C) and Monocytes (D) in Benzene Induce Leukemic wistar rats

Effect of *Acacia nilotica* extracts on lactate dehydrogenase (LDH), Aspartate (AST), Alanine phosphatase (ALP) and Alanine transaminase (ALT) in Benzene Induce Leukemic wistar rats.

Figure 3A shows a significantly ($p < 0.01$) high amounts of lactate dehydrogenase (LDH) in the positive control compared to the negative control. The standard control also had a significantly ($p < 0.01$) low amount of LDH compared to the positive control but same range with that of negative control. Groups treated with *Acacia nilotica* (7.5, 10.0 and 12.5 mg) were significantly ($p < 0.05$) lower compared to positive control group, though 10.0 and 12.5 mg *Acacia nilotica* showed severe toxicity. As shown in figure 3B there was significantly ($p < 0.05$) higher activities of AST in the positive control compared to the negative control. The groups treated with 7.5, 10.0, 12.5 mg *Acacia nilotica* had significantly ($p < 0.05$) lower activities of AST compared to the standard and positive controls, showing anti-leukemic activities. As shown in figure 3C, there was significantly ($p < 0.001$) low activities of ALP in the positive control compared to negative control. The result in standard control showed significantly ($p < 0.05$) higher activities of ALP compared to positive control. The groups treated with 7.5, 10.0, 12.5 mg *Acacia nilotica* had significantly ($p < 0.001$) low activities of ALP compared to the standard control, showing toxicity. As shown in figure 3D, the activities of ALT in the positive control were significantly ($p < 0.01$) higher compared to both negative and positive controls. There were significantly ($p < 0.05$) low differences between the standard control and the group treated with *Acacia nilotica* (7.5, 10.0 and 12.5 mg)

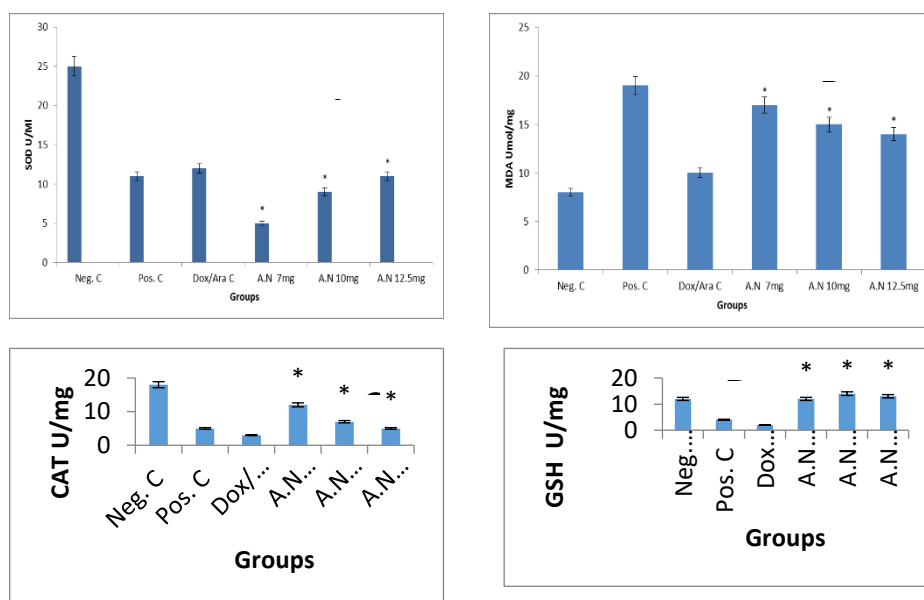


Results represented as mean $\pm$ SD (n=5). Symbols represent the level of significance *= $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

Fig. 3 Effect of *Acacia nilotica* extracts on lactate dehydrogenase (LDH), Aspartate (AST), ALP and Alanine transaminase (ALT) in Benzene Induce Leukemic wistar rats.

Effect of *Acacia nilotica* extracts on Superoxide dismutase (SOD), Malondialdehyde (MDA), Catalase (CAT), reduced Glutathione (GSH) in Benzene Induce Leukemic wistar rats

The activity of SOD was significantly ($p < 0.05$) lower in the positive control compared to the negative control group figure 4A. The concentrations of MDA were significantly ($p < 0.05$) higher in the positive control compared to the negative control. The standard control was in the same range with the negative control. All the treatment groups (7.5, 10.0 and 12.5 mg) were significantly ($p < 0.05$) lower in a dose dependent manner compared to the positive control group as shown in figure 4B. The concentration of GSH in the positive control was lower compared to the negative control. There was further depression in the activities of GSH in the standard control which may be due to toxicity of the drug. Experimental groups treated with 7.5, 10.0, 12.5 mg, *Acacia nilotica* extract were significantly ($p < 0.05$) higher compared to the standard control group as shown in figure 4D.



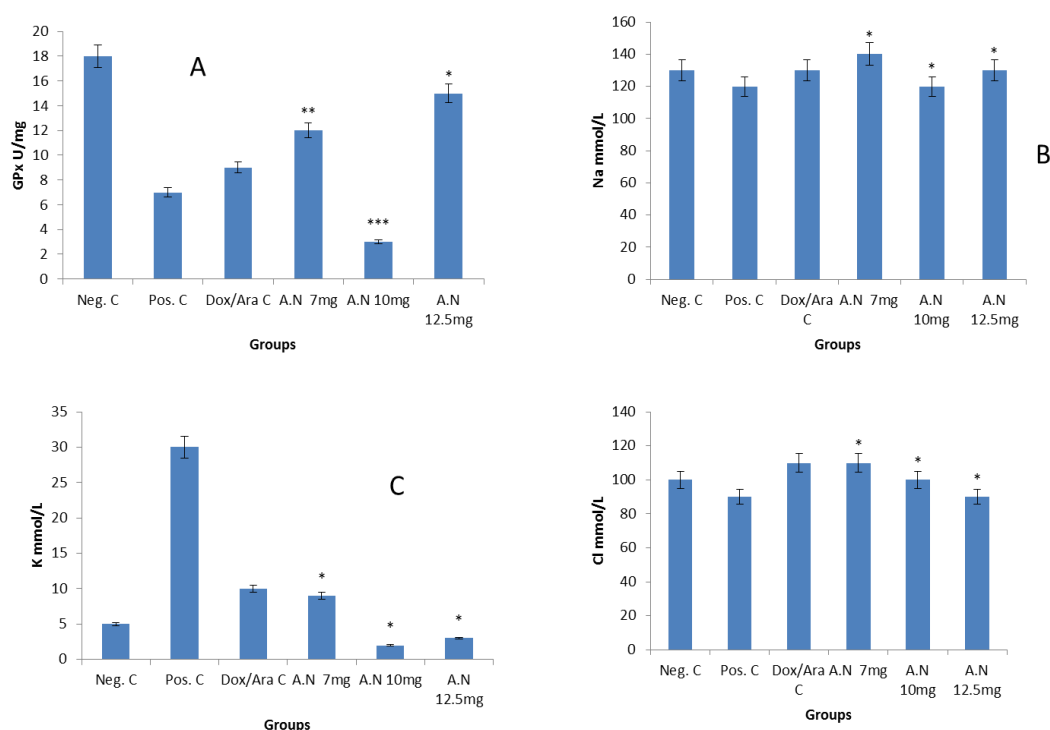
Results represented as mean $\pm$ SD ($n=5$). Symbols represent the level of significance * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

Fig 4 Effect of *Acacia nilotica* extracts on Superoxide dismutase (A), Malondialdehyde (B), Catalase (C), reduced Glutathione (D) in Benzene Induce Leukemic wistar rats

Effect of *Acacia nilotica* extracts on Glutathione Peroxidase (GPx), reduced Sodium ion (Na^+), on reduced Potassium ion (K^+) and reduced Chloride ion (Cl^-) in Benzene Induce Leukemic wistar rats

The activity of GPx in the positive control was significantly ($p < 0.05$) lower compared with that of the negative control. The activity of the standard control was slightly higher than that of the positive control. The groups treated with 7.5, 10.0 and 12.5 mg *Acacia nilotica* extracts are significantly ($p < 0.05$) higher (in a dose dependent manner) compared with the positive control as shown in figure 5A. Figure 5B showed a significantly ($p < 0.05$) lower level

of Na^+ ions in the positive control compared to the negative control. The standard control is significantly ($p>0.05$) higher than the positive control, restoring Na^+ ions back to the negative control. All the treatment groups with 7.5 and 12.5 mg except 10.0 mg of *Acacia nilotica* extracts were significantly ($p>0.05$) higher than the positive control. The concentration of potassium ion was significantly ($p<0.05$) high in the positive control compared to the negative control. The concentration in standard control was significantly ($p<0.05$) lower compared to the positive control. All the treatment groups (7.5, 10.0 and 12.5 mg) *Acacia nilotica* extracts significantly ($p<0.05$) had low concentrations of potassium ion figure 5C There is no significant difference between the positive control to the negative control. There is also no significant difference across the treatment groups with both the positive and standard controls figure 5D



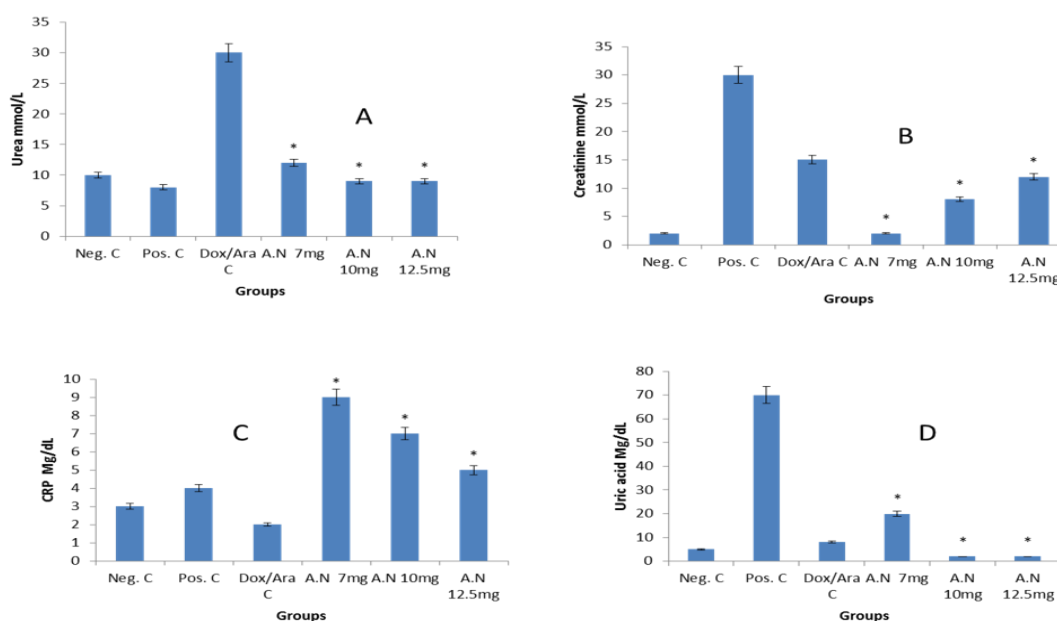
Results represented as mean $\pm$ SD ($n=5$). Symbols represent the level of significance *= $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

Fig 5 Effect of *Acacia nilotica* extracts on Glutathione Peroxidase (A), reduced Sodium ion (B), on reduced Potassium ion (C) and reduced Chloride ion (D) in Benzene Induce Leukemic wistar rats

Effect of *Acacia nilotica* extracts on Urea, Creatinine, C-reactive Protein and Uric acid Concentration in Benzene Induce Leukemic wistar rats

The concentrations of urea were significantly ($p<0.001$) higher in the standard control compared with the negative control as seen in fig 6A. The standard control is significantly ($p<0.01$) higher compared to positive control which may be due to cytotoxic effect of the

drug. There were significantly ($p < 0.05$) low levels of urea in the groups treated with *Acacia nilotica* (7.5, 10 and 12.5 mg) compared to the standard control group bringing the levels in the same range with that of the negative control. Creatinine concentration in the positive control was significantly ($p < 0.001$) high compared to the negative control. The standard control was significantly ($p < 0.05$) lower than the positive control. The groups treated with 7.5 mg *Acacia nilotica* was significantly ($p < 0.001$) lower than the positive control restoring creatinine to normalcy (Figure 6B). As shown in figure 6C, there was no significant difference between the levels of C-reactive Protein between the positive and negative controls. The standard control had a low C- reactive protein compared to the positive control. All the treatment groups 7.5, 10.0, 12.5 mg *Acacia nilotica* extract had significantly ($p < 0.05$) high CRP concentrations compared to the standard control. The concentration of uric acid was significantly ($p < 0.001$) high in the positive control compared to the negative control group. The standard control was significantly ($p < 0.05$) lower compared to the positive control almost restoring uric acid to normalcy. All the treatment groups with the exception of 7.5 mg *Acacia nilotica* were significantly ($p < 0.001$) lower than the positive control with some showing toxicity as shown in figure 6D.



Results represented as mean $\pm$ SD ($n=5$). Symbols represent the level of significance *= $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

Fig 6: Effect of *Acacia nilotica* extracts on Urea (A), Creatinine (B) C-reactive Protein (C) and Uric acid (D) Concentration in Benzene Induce Leukemic wistar rats

Discussion

Acacia nilotica is among the safest natural, versatile compounds used today, with no significant toxic or adverse effects ever being associated with its vast applications (Ali *et al.*, 2012).

The phytochemical analysis in this study showed that *Acacia nilotica* contains phytochemical compounds such as Alkaloids, Tannins and Phytosterol in notable concentrations, the result is similar to the previous findings of Deshpande, 2013; Banso, 2009 and Okoro *et al.*, 2014. Presence of alkaloids and the oxidant generation potential, or a combination of these mechanisms made the plants to have potential therapeutic properties (Deshpande, 2013 and Banso, 2009). Plant that contains tannins could be an excellent source of natural antioxidants (Wong *et al.*, 2014). Antioxidant and anticancer potential could be due to the presence of tanins, alkaloids or phenolic compounds (Kalaivani and Mathew 2010).

Plate **1A** is a blood smear slide prepared from a negative control. Red blood cells are predominant, White blood cells (WBC) are also spotted looking bluish due to the kit-ral staining colour, careful observation revealed the presence of platelets. Plate **1B** was prepared from a positive control group, the slide was prepared after six weeks of inducing leukemia using benzene 48 hourly. On Plate **1B**, the WBCs are larger and more in number due to sudden and uncontrolled growth of immature haemapoeitic cells at the expense of normal blood cells just as observed by many researchers such Soni and Krishnamurthy, 2013; Sanal *et al.*, 2015; Olufemi *et al.*, 2017; Kabeel *et al.*, 2018; 2022. Here the RBC and Platelets were not as much as in Plate **1C**. Plate **1C** was prepared after the leukemic rats were treated with a standard drug (doxorubicin/Ara-C). WBCs, RBCs and Platelets reduced drastically, this is because the standard drug is cytotoxic and not selective in the destruction of cells. Plate **1D**, **E** and **F** were prepared after treating leukemic rats with *Acacia nilotica* extract at 7.5, 10.0 and 12.5 mg respectively. All rats treated with 2.5 and 5.0 mg died before the treatment ends, showing that the therapeutic effects of the lower doses were not potent. Higher concentrations of 10.0 and 12.5 mg were added, and compared as shown above. The cells observed on the slides showed reduction in the WBC both in number and size (dose dependent) compared with the positive control indicating that the plant contains some anti-leukemic properties.

The groups treated with 7.5, 10 and 12.5 mg *Acacia nilotica* showed a significantly ($p < 0.05$) higher level of WBC compared to the control groups. The major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms in immune system. Thus, animals with low white blood cells or high abnormal WBC (as in case of leukemia) are exposed to high risk of disease infection, while those with high counts of healthy blood cells are capable of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases and enhance adaptability to local environment and disease prevalent conditions. *Acacia nilotica* is among the safest natural, versatile compounds used today, with no significant toxic or adverse effects ever being associated with its vast applications (Ali *et al.*, 2012).

Acacia nilotica had significantly high level of white blood cell (leucocyte) count only at 7.5, 10 and 12.5 mg of treatment. An elevated leucocyte count is not only an indication for infection or stress but also denotes a reaction to a therapeutic agent especially steroids that causes increased leucocyte production. This result suggests that *Acacia nilotica* may have

the capacity to increase white blood cell production and could be attributed to the Phyto-steroidal constituents present in the extracts as seen in Table 1 above. Animals with elevated leucocyte count can produce antibodies in the process of phagocytosis and as such are conferred with high degree of immunity. Note also that WBC count may remain high for non-malignant reasons after therapy such as infection (due to low active WBC), inflammation (arising both from direct cytotoxic effects on malignant cells and from immune-mediated responses to novel treatment modalities), reactive leukocytosis, steroid use (common in leukemia regimens and causes demargination of neutrophils, artificially raising WBC counts), marrow recovery or rebound after chemotherapy, stress from therapy which can also temporarily elevate WBC, marrow regenerating healthy cells or treatment side-effects (Kornberg and Polliack 1980). This apparent paradox is explained by the different biological processes reflected by these parameters.

As shown in figure 1B, RBC were significantly ($p < 0.05$) lower in the positive control compared with the negative control group, this may be due to the proliferation of immature blasts that hinders the production of normal blood cells, RBC inclusive. The groups treated with 12.5 mg *Acacia nilotica* were all lower than the standard control group which may be due to cytotoxicity of the plants extracts as seen in (Foo *et al.*, 2011).

Figure 1D showed that platelets counts were significantly ($p < 0.001$) low in the positive control compared to the negative control, this is due to the increase in the production of abnormal WBC which crowd out other cells platelet inclusive. The standard control is higher than the positive control which is a sign that there is partial restoration of platelet. The groups treated with *Acacia nilotica* have higher level of platelets than showing that the cytotoxicity of *Acacia nilotica* is low. Neutrophils work to fight against infection by either consuming the foreign body through phagocytosis or releasing destructive enzymes (N.C Institute, 2021). The groups treated with 7.5, 10 and 12.5 mg *Acacia nilotica* as the level of neutrophils significantly ($p < 0.05$) become higher compared to the standard control group. The high level of neutrophils in the groups treated with *Acacia nilotica* maybe due to the machine picking and counting all blasts as neutrophils since the nucleus at early stage looks same. Other factors responsible for the high level may be tissue damage, inflammation and malignant reasons just as discussed above under WBC (Figure 2A). Eosinophils are infection fighting cells that play an important role in inflammation and perform similar functions as neutrophils, though they have a higher concentration of granules, making the cytoplasm of the cell appear pink when stained (Kebe *et al.*, 2025). The groups treated with *Acacia nilotica* (7.5, 10, 12.5 mg) were significantly ($p < 0.05$) higher than the standard control. Benzene metabolism, chronic inflammation and oxidative stress/immune deregulation might have contributed to the different levels of eosinophils in different plant groups (Figure 2B). Lymphocytes are found in the blood and lymph tissue and can either make antibodies or help kill tumor cells. They contain a single large nucleus which is usually round with very scanty cytoplasm. The groups treated with *Acacia nilotica* (7.5, 10, 12.5 mg) all had a significant ($p < 0.05$) lower level of lymphocytes compared to positive control group. The low levels may be associated to depressed leucopoiesis in the bone marrow which may have

consequential effects on the immune system and phagocytic activities of the blood cells of the animals (figure 2C). Monocytes function by developing into macrophages which phagocytose dead cells and bacteria. They have large, irregularly shaped nuclei and some small granules in the cytoplasm. Experimental groups treated with *Acacia nilotica* (7.5, 10, 12.5 mg) were significantly ($p < 0.05$) higher compared to the standard control group, the result co-relates with the findings of Foo *et al.* (2012). The extract showed therapeutic properties and was more cytotoxic compared to the standard drug (figure 2D). The levels of Lactate dehydrogenase (LDH) enzymes are highly elevated in the positive control possibly due to alterations in carcinogenic status that can lower the conversion rate of lactate to pyruvate ending with increased nicotinamide adenine dinucleotide (NAD⁺) that can interact with metabolic pathways of carbohydrate, this is called Warburg effect. A high glycolytic activity is thought to increase the level of LDH which is likely due to cancer development. The levels of serum LDH are used to diagnose a variety of cancers including oral, laryngeal and breast cancer. From a clinical point of view, the variation in the levels of lactate with time reflects directly its production changes. Elevated levels of lactate give a clue for circulatory alterations, while its lowered levels pointed to improved circulation. Groups treated with *Acacia nilotica* extracts are significantly ($p < 0.05$) lower compared to the positive group, which shows that the excessive LDH brought by leukemia burden has been eliminated due to the plant extracts therapeutic effects. ALT catalyzes the transfer of an amino group from L-alanine to 2-oxoglutarate to form pyruvate and L-glutamate. An increase in pyruvate concentration corresponds with the levels of AST and ALT activities. The pyruvate concentration is determined spectrophotometrically in the form of hydrazone, which is produced by reaction with 2,4-dinitrophenylhydrazine in an alkaline medium. The apparent reduction in aminotransferase in experimental groups treated with *Acacia nilotica* may be related to decreased pyridoxal phosphate, the prosthetic group for aminotransferases, resulting in an increase in the ratio of apoenzyme to haloenzyme. Alkaline phosphatase (ALP) is an enzyme found in several tissues throughout the body. The highest concentrations of ALP are present in the cells that comprises of bone and the liver. Elevated levels of ALP in the blood are most caused by liver disease or bone disorders. The activities of ALP in the groups treated with 7.5, 10 and 12.5 mg *Acacia nilotica* extract had significantly ($p < 0.001$) low activities of ALP compared to the standard control. This may be due to cytotoxicity and the effect of the extracts on the activities of the enzyme. The results obtained in this study are similar to studies done by Rosenblum and Petzold (1975); Valentine *et al.* (1952); Wiltshaw and Maloney (1955). Liver transaminases, mainly alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are aggregated in the cytosol of hepatocytes, these enzymes are normally detectable in the serum at low concentrations. As shown in figure 3D, the activities of ALT in the positive control are significantly ($p < 0.01$) higher compared to that of negative control. The activities of the enzyme in all the groups treated with *Acacia nilotica* (7.5, 10.0 and 12.5 mg) were significantly ($p < 0.05$) lower than the standard control. The elevated alanine transaminase (ALT) levels observed in this study following leukemia treatment are consistent with

previous reports of hepatocellular injury secondary to chemotherapeutic and targeted agents (Kamal *et al.*, 2019). Similar post-treatment ALT elevations have been widely documented in the literature, reflecting the hepatotoxic effects of several drugs used in leukemia management. The present findings align with existing evidence that high ALT levels after leukemia therapy are multifactorial, often involving drug-induced liver injury, viral reactivation, and metabolic or transfusional factors (Kamal *et al.*, 2019; Hwang, 2020; Wang 2021; U.S. National Library of Medicine).

Superoxide dismutase (SOD) is a group of key enzymes functioning as the first line of antioxidant defense by virtue of their ability to convert highly reactive superoxide radicals (dismutation) into hydrogen peroxide and molecular oxygen. Groups treated with *Acacia nilotica* (12.5 mg) had significantly ($p < 0.05$) lower levels of SOD compared to the standard control group in a dose dependent manner. The observed decline in SOD after leukemia treatment reflects both drug-induced oxidative burden and suppression of endogenous antioxidant systems. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells (Gawel *et al.*, 2004). It is one of the radical oxidative markers. Increase in free radical causes overproduction of MDA. Level of MDA is commonly known as a marker of oxidative stress and the antioxidant status in a cancerous patient (Gawel *et al.*, 2004). It is not an antioxidant but used to access the status of endogenous antioxidants such as SOD (Gawel *et al.*, 2004). The concentrations of MDA were significantly ($p < 0.05$) higher in the positive control group compared to the negative control group as seen in figure 4.15. This is like the research done by Ahmed *et al.* (2008) who showed that the level of MDA is higher in leukemia patients compared with non-leukemic patients (Gawel *et al.*, 2004). This indication that the level of free radicals is high for the antioxidants in the leukemic rats leading to high level of oxidative stress. The groups treated with *Acacia nilotica* (7.5, 10.0 and 12.5 mg) were able to ameliorate the level of MDA lower than that of positive control. This is in conformity with Ali *et al.* (2020) who showed in their work how elevated level of MDA and superoxide production became lower significantly when treated *Acacia nilotica* with extracts. Catalase is a tetrameric porphyrin containing enzyme that is located mainly in peroxisomes. It catalyzes the conversion of H_2O_2 to water and molecular oxygen in two steps. The activities of catalase remained significantly ($p < 0.05$) high in groups treated with 7.5, 10, 12.5 mg *Acacia nilotica* extract. The result co-relates with that of Kidson (1962) who showed that leukemic patients had lower levels of catalase as seen in this study (Kennedy *et al.*, 202). This shows that treatment with the *Acacia nilotica* extract were able to restore catalase close to normal level. Glutathione [GSH is an antioxidant responsible for the elimination of damaging lipid peroxides, and other ROS species (Kennedy *et al.*, 202). The reduced form (GSH) is biologically active. GSH deficiency or a change in GSH/GSSG ratio increases the vulnerability of cells to oxidative stress, inflammation, and tumor progression. However, elevated GSH levels increase antioxidant capacity and resistance to oxidative stress as is evident in many tumors (Kennedy *et al.*, 202). The concentrations of GSH are significantly ($p < 0.05$) lower in positive control compared with the negative control which is due to the burden of oxidative stress,

inflammation and ROS due to leukemia. The concentrations of GSH are significantly ($p < 0.05$) lower in positive control compared with the negative control which is due to the burden of oxidative stress, inflammation and ROS due to leukemia. On the other hand, the concentration of GSH is significantly ($p < 0.05$) higher across all the experimental groups *Acacia nilotica* (7.5, 10, 12.5 mg), compared to the positive control groups as shown in figure 4D. These results proved that *Acacia nilotica* extract was able to elevate the GSH level from where it was reduced due to leukemia induction to a higher level. Higher level means more antioxidant capability which translates to improving the disease condition leading to healing. Ali *et al.* (2020) in their work showed that *Acacia nilotica* has the ability to increased GSH in a decreased state due to its amino acids contents that boost the antioxidant capacity of the plant (Ali *et al.*, 2012). The low level in the GSH in the group treated with standard drug may be due to the cytotoxicity of the drug. Glutathione peroxidases GPx are members of a multi-enzyme family that catalyze the reduction of H_2O_2 , organic hydroperoxides and/or lipid hydroperoxides to generate their corresponding lipid alcohols and water at the expense of oxidation of two molecules of GSH. During the reaction GSSG (oxidized glutathione) is generated (Kennedy *et al.*, 2020). The activities of GPx are significantly ($p < 0.05$) lower in the positive control compared to the negative control, obviously due to burdens related to leukemia induction. The activity of the standard control was slightly higher than that of the positive control showing therapeutic activities. All the groups treated with *Acacia nilotica* (7.5, 10.0 and 12.5 mg) had significantly ($p < 0.05$) higher levels of GPx compared to the positive control (in a dose dependent manner) which imply that oxidative stress has been lowered.

Figure 5B showed that the concentration of sodium in the positive control is significantly ($p < 0.05$) lower compared to the negative control. The standard control is significantly ($p < 0.05$) higher than the positive control, restoring Na^+ ions back to the negative control range. The concentrations of sodium in the groups treated with 7.5 mg *Acacia nilotica* were all significantly ($p < 0.05$) higher compared to the positive control. This can be interpreted to be that the plants extract just as the standard drug has therapeutically elevated the concentrations of sodium that was reduced because of the leukemia burden. The concentration of potassium ion in the positive control is significantly ($p < 0.05$) higher compared to the negative control, this may be due to both benzene metabolism and leukemia burden. Treatment groups were all significantly ($p < 0.05$) lower than the positive control. This signifies the plant extracts were able to restore potassium concentration because of their therapeutic properties. Figure 5C show no significant difference in the level of chloride ions between the negative and positive control, which means that leukemia has no significant effect on chloride. There is however significantly ($p < 0.05$) high level of chloride ions in the group treated with 7.5 mg gum Arabic compared to the control groups as shown in figure 5D. The reason for this requires further studies. The concentrations of urea were significantly ($p < 0.001$) higher in the standard control compared to the negative control as seen in figure 6A. The standard control is significantly ($p < 0.01$) higher compared to positive control which may be due to cytotoxic effect of the drug. In the groups treated

with 7.5, 10 and 12.5 mg *Acacia nilotica* the concentrations of urea were restored to that of the negative control with the lowest concentration to be optimal, showing significantly ($p < 0.05$) low levels of urea in comparison to that of standard control. This suggests that the plant extract can stabilize the concentration of urea to normal. Creatinine concentration was significantly ($p < 0.001$) high in the positive control compared to that of negative control as depicted in figure 6B. It is normal for creatinine concentration to be high in leukemic condition but the highly excessive rise may not be explained in this study but subject to further investigation. The standard control was significantly ($p < 0.05$) lower than the positive control, but significantly high compared to the negative control group. Elevated creatinine levels after chemotherapy are a frequent finding and often indicate renal impairment. Many chemotherapeutic agents, are known to cause dose-dependent nephrotoxicity, which reduces glomerular filtration rate (GFR) and leads to increased serum creatinine (Kintzel, 2001). The accumulation of creatinine reflects decreased renal clearance rather than overproduction. Furthermore, tumor lysis syndrome (TLS) following rapid cancer cell destruction can precipitate acute kidney injury due to uric acid and calcium phosphate crystal deposition within renal tubules, further elevating creatinine (Howard *et al.*, 2011). Dehydration, sepsis, or the concurrent use of nephrotoxic antibiotics and contrast agents can worsen this effect (Perazella, 2012). Hence, regular monitoring of serum creatinine during and after chemotherapy is essential for early detection of renal dysfunction and appropriate treatment adjustments. The groups treated with 7.5 mg *Acacia nilotica* had significantly ($p < 0.001$) lower levels of creatinine than that of positive control showing restoration of creatinine concentration back to the normal level, this indicates that *Acacia nilotica* possess therapeutic properties capable of removing excessive creatinine in circulation. There were significantly ($p < 0.05$) high levels of C-reactive protein in the treatment groups compared to the standard control group. This means the *Acacia nilotica* extract induces slight inflammation. Inflammation is a common but complex consequence of leukemia therapy, arising both from direct cytotoxic effect on malignant cells and from immune-mediated responses to novel treatment modalities. Understanding these inflammatory pathways is essential for distinguishing treatment-related complications from infectious processes and for optimizing supportive care in affected (Sonis, 2004). Thus, inflammation after leukemia treatment arises not only as a complication of cytotoxic therapy (direct tissue damage and cytokine release) but also as an intended immune activation in modern immunotherapies. Monitoring of inflammatory markers such as CRP, ferritin, and IL-6 is essential to distinguish between infection and therapy-induced inflammation. Uric acid comes mainly from cell breakdown, in leukemia it is primarily influenced by lysis of leukemic cells, purine metabolism, kidney excretion, and the use of uric acid-lowering therapies (Pession and Melchionda, 2009; Solh *et al.*, 2013; Haen *et al.*, 2013). The concentration of uric acid was significantly ($p < 0.001$) high in the positive control compared to the negative control, suggesting that the concentration of serum uric acid (sUA) in the positive control is excessively high. This is a common situation in untreated leukemia due to rapid tumor cell turnover and the release of large amounts of nucleic acids

due to lysis which metabolized to uric acid. The group treated with standard drug had a significantly ($p < 0.05$) lower concentration of sUA compared with that of the positive control and almost same level with that of negative control, which means the standard drug was able to restore UA to normalcy. All the treatment groups 7.5, 10, 12.5 mg *Acacia nilotica* extract showed significantly ($p < 0.05$) lower uric acid concentrations compared to the positive control group as shown in figure 6D. After effective treatment (or once remission is achieved), there is less leukemic cell destruction, so uric acid levels (Pession and Melchionda, 2009; Solh *et al.*, 2013; Haen *et al.*, 2013).

Conclusion

Medicinal plants have demonstrated significant application in the pharmaceutical industry in the production of drugs that combats different disease. This may be linked to their availability and less toxicity to the overall physiology of humans. This research was designed to evaluate the potentials of *Acacia nilotica* extract in leukemia. The plant alleviated the haematological malignancy, liver toxicity and renal disturbance caused by benzene, this effect may be attributed to the plants antioxidant capability and phytochemicals present in the plant extract. Therefore, *Acacia nilotica* may be used as complementary medicine in leukemia conditions and oxidative stress.

Recommendations

Quantitative phytochemical analysis of *Acacia nilotica* should be carried out to determine which bio-active compound is more in each and how such bio-active compounds play the anti-leukemic activities. Determine the LC₅₀ of the plants so that it can help to determine the doses that will give optimal results.

Acknowledgment

Gratitude goes to Prof. Moses Zira Zaruwa for his guidance, supervision and review of this article.

Ethical approval

Ethical approval was obtained from Nasarawa State University Keffi animal care and use research committee (NSUK-ACUREC) with approval number NSUK-ACUREC/BCH/23/14-24/11/2023.

Conflict of interest

The authors declare that they have no conflicts of interest to this work

Limitations

This study focused on the *in-vivo* anti-leukemic potentials of *Acacia nilotica*. A major limitation was failure to carry out genetic testing such as BCR-ABL-1 which is a major

biomarker in evaluating the presence and prognosis of leukemia, another limitation was small experimental size.

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