

Chemoprotective and Nephro-Protective Effects of *Manihot esculenta* Methanolic Root Extract in Benzene-Induced Leukemia in Mice

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Abstract

Leukemia is a disease of the blood and bone marrow which is characterized by accumulation of white blood cells in the bone marrow and intercalation into peripheral blood causing haematological and renal disorders. The purpose of this research was to evaluate the performance of *Manihot Esculenta* methanolic roots extract on haematological and renal alterations in leukemia mice model. Leukemia was induced in mice with 2.0 mL of benzene solution intravenously from the tail vein 48 hourly for three consecutive weeks. The mice were divided into 6 groups; group 1-normal control received commercial feed and water, group 2- benzene positive control received feed+ water + benzene, group 3- chemotherapeutic drug control received commercial feed+ water+ benzene+ doxorubicin/ cytarabine drug regimen while group 4, 5 and 6 received feed + water + benzene and treated with 5.0 mg/kg, 10 mg/kg and 15 mg/kg *Manihot esculenta* roots extract. Blood samples were collected to assay for haematological indices, c-reactive proteins concentration, electrolytes parameters and blood microscopy. Blood microscopy result presented blast cells in benzene group, while haematological and electrolytes parameters showed a significant $p < 0.05$ increase in phosphate, white blood cells, lymphocytes, uric acid, potassium, sodium and decrease in calcium, platelets and red blood cells in benzene positive control group compared to normal control group which had normal concentrations. Treatment with *Manihot Esculenta* extract and doxorubicin/ cytarabine chemotherapeutic drug regimen significantly $p < 0.05$ reversed haematological and electrolytes parameters close to normal ranges. Therefore, *Manihot Esculenta* roots may be used for herbal therapeutic development or prophylaxis to cushion the effect of leukemia on kidney function and haematological indices. Although this research had limitations in carrying genetic testing like BCR-ABL1 gene therefore we recommend genetic and in-silico studies to assess the druggability of bioactive compounds in *Manihot esculenta* to specific genes or proteins common in leukemia.

Keywords: Leukemia, Nephro-Protection, Chemo-Protective, Benzene, *Manihot esculenta*.

Introduction

Leukemia is a group of hematological malignancy which presents an uncontrolled, unregulated and rapid proliferation of leukemic cells resulting in replacement of normal haematopoietic cells by abnormal proliferating cells in the bone marrow and spilling over peripheral blood (Shuchismita *et al.*, 2022). The bone marrow is a soft and versatile tissue which is the site of haematopoiesis, producing millions of blood cells daily (Yadav *et al.*, 2023). Leukemia is either acute or chronic depending on the cell line (myeloid or lymphoid) it arises from therefore it is classified into four broad types – acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL) (Karunarathna *et al.*, 2012). CLL is the most prevalent type of leukemia in the western world, in CLL, monoclonal B-lymphocytes accumulates in blood, bone marrow and lymph nodes (O'Donnell *et al.*, 2023). A combination of genetic and environmental factors is associated to leukemogenesis these factors include smoking, ionizing radiation, exposure to petrochemicals such as benzene, prior chemotherapy and down syndrome (John and Hutter, 2010). Benzene and its metabolites cause chromosomal aberrations in the peripheral blood lymphocytes of chronically exposed humans (McHale *et al.*, 2012).

Medicinal plants have been used as medicine by mankind to treat health-threatening diseases and still popular to obtain new drug candidates as it is the oldest medical practice for humans (Mahady, 2001). *Manihot esculenta* is one of the most consumed plants in Africa, its roots and leaves are used in Nigeria for the treatment of ringworm, tumor, conjunctivitis, sores and abscesses (Miladiyah, 2011). Its extract also showed antioxidant and antiradical properties in a model of a complete lipid peroxidation, leaves of *Manihot esculenta* were reported to have anti-hemorrhoid, anti-inflammatory and anti-microbial activity (Okpuzor and Oloyede, 2009 ; Popoola and Akintokun, 2007). The leaves have also been used against many disorders, such as rheumatism, fever, headache, diarrhea and loss of appetite (Kale and Bahekar, 2013) A study by Ayodeji, 2015 opined that *Manihot esculenta* is rich in macro and micro-nutrients and therefore commonly used as a staple food. In addition, the plant contains a number of antioxidant compounds, namely α -carotene (around 23-86 mg/100g), vitamin C (1.7-419 mg/100g), vitamin A, anthocyanins (flavonoids), saponins, steroids, and glycosides which are necessary for regulation of normal homeostasis (Chavez *et al.*, 2000)

Diagnosis of leukemia is done typically by blood tests such as complete blood count, peripheral blood microscopy to ascertain the presence of blasts cells and bone marrow biopsy (Zhou *et al.*, 2021). Although, acid base balance disorders and electrolytes abnormalities have been recorded in patients with leukemia (Filippatos *et al.*, 2005). Alterations in electrolytes homeostasis may lead to physiological disorders (Ujong *et al.*, 2022). These disorders may result to fatal complications and sudden death due to malignant arrhythmias and organ failure (Filippatos *et al.*, 2005). Electrolytes imbalance in leukemic patients is also as a result of tumor lysis syndrome (TLS) which is an oncogenic emergency enhancing the lysis of tumor cells to release electrolytes, metabolites and enzymes spontaneously due to tumor burden or chemotherapy (Ahmed *et al.*, 2019;

McGhee-Jez *et al.*, 2018; Wierda *et al.*, 2019). In TLC, electrolytes such as sodium, potassium, phosphorus and chlorine are released in high concentration while calcium is released in relatively low concentration. Metabolites like uric acid which is released in high concentration due to nucleic acid (purine) catabolism, lactate dehydrogenase enzyme is also released in high concentration. These metabolites, enzymes and electrolytes are prognostic biomarkers useful to monitor the progression of leukemia (Boles *et al.*, 1984; Kanfer *et al.*, 1979; Howard *et al.*, 2011). Therefore, health practitioners should be watchful on electrolytes concentrations before the initiation of chemotherapy regimen and during treatment protocol.

Subsequent works has been done on medicinal plants which have anticancer activity although there is limited research on the chemotherapeutic potentials of *Manihot esculenta* in leukemia. Therefore, the aim of this research was to evaluate the chemo-protective and nephron-protective effects of *Manihot esculenta* roots extract on renal and haematological parameters in benzene- induced leukemia mice model.

Research Methodology

Preparation of plant material

The roots of *Manihot esculenta* were purchased from local marketers of old market Keffi Nasarawa State. The roots were identified in the department of Plants science and Biotechnology Nasarawa State University Keffi and a voucher No. 6 was assigned. The roots peels were removed from the roots. The roots were washed with distilled water, sliced to smaller sizes and air dried under laboratory conditions for two weeks in the Department of Biochemistry, Nasarawa State University, Keffi. The dried roots were grinded to pass a 0.5 mm sieve to provide a greater surface area, 20 g of the dried powder was filled in the porous cellulose thimble and subjected to soxhlet extraction using methanol for 12 hours at 100°C. The extract was evaporated in a rotary evaporator (RE 300) at 60°C.

Acute Toxicity of *manihot esculenta* methanolic extract

Per oral (P.O) route was used to determine the acute toxicity (LD₅₀) of *Manihot esculenta* roots extract in mice of both sexes following the procedure of Isah *et al.*, (2007). 15 mice were divided into 3 groups of 5 each used for assessing LD₅₀. Varying doses of the extract ranging from 1000-3000 mg/kg body weight was administered to the mice as single dose. After the administration of the extract, the mice were observed at 6 hourly intervals for the next 48 hours to observe any death or changes in general behaviour and other physiological activities (Ogbonnia *et al.*, 2009).

Weight management of the plants' extracts

Body weight of all group mice was assessed every two weeks throughout the experimental period to evaluate the weight management efficacy of *Manihot esculenta* roots extract (Liu *et al.*, 2022).

Experimental animals

A total of 45 mice weighting 150-200 g were used for the study, 15 mice for acute toxicity test and 30 for *in-vivo* experimental design. The mice were acclimatized to laboratory conditions for two weeks before the beginning of experiment (Zaruwa *et al.*, 2016). The mice were maintained at room temperature and with a 12 h light/12h dark cycle and allowed *ad libitum* access to feed and water (Ola *et al.*, 2021). 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.

In-vivo studies

Experimental design

A Completely Randomized Design (CRD) was used with 5 replicates assigned to each group. 30 mice of both sexes were used for the experiment weighing 150-200 g and divided into 6 groups as follows:

GROUPS	TREATMENT		
(1) CONTROL	feed + water		
(2) POSITIVE CONTROL	feed + water +	benzene	
(3) STANDARD DRUG	Feed + water+	Benzene +	Doxorubicin /Ara C
(4) GROUP 4	Feed + water+	Benzene +	2.5 mg / kg (M.E)
	Feed + water +	Benzene +	5.0 mg/ kg(M.E)
(5) GROUP 5	Feed + water +	Benzene +	10 mg / kg (M.E)
(6) GROUP 6			

Group 1: control (received feed +water), Group 2: Benzene control (received feed + water + benzene), Group 3: Dox/Ara C (received feed + water + Benzene + doxorubicin D KLAB KD-349/cytarabine or Ara C zydus celexa MH/102580A standard drug regimen), Group 4, 5 and 6 (received feed + water + Benzene) and treatment with 2.5 mg/kg, 5.0 mg/kg and 10 mg/kg of *Manihot esculenta* roots extract respectively by oral gavage daily for 2 weeks.

2.5.2 leukemia induction

Benzene (99%) with Cat No. MCR-28775 purchased from MOLYCHEM India was used to induce leukemia in mice by intravenous injection of 0.2 mL of solution (Akanni *et al.*, 2017). 1 mL of the benzene was diluted in 5 mL of distilled water and 4 mL of propanol (1:5:4 of benzene /distilled water / propanol v/v) to make up the solution of which 0.2 mL was administered intravenously through the tail vein every 2 days for three consecutive weeks (Akanni *et al.*, 2017). After complete induction, blood tests were carried out to ascertain the presence of leukemia in selected mice. Leukemia was allowed to develop in those mice before commencement of treatment protocol.

Blood samples collection

The mice were sacrificed at the end of the experimental process. Blood samples were collected by bleeding the jugular vein and dispensed into ethylene di-amine tetra acetic acid (EDTA) and lithium heparinized tubes. The samples were labelled, some centrifuged and plasma collected and stored appropriately for biochemical analysis.

Peripheral blood microscopy examination

Blood microscopy was carried out following the procedure of Bain *et al.*, 2006. Blood samples of different groups were stained on a slide with Kit RAL 555 (Ref. 361550) giemsa and viewed under a triocular microscope (Swift SW350T), to ascertain the presence of blast cells and morphology of blood cells (Bain *et al.*, 2006).

Haematological parameters analysis

Hematological analyzer (Celltrac G MEK- g100) which uses flow cytometry principles to count blood cells such as RBC, WBC, haemoglobin (Hb), packed cell volume (PCV), platelets, mean corpuscular haemoglobin (MCV), haematocrit (HCT), mean corpuscular haemoglobin concentration (MCHC) and differential white blood cells (eosinophil, basophils, neutrophils monocytes and lymphocytes) with appropriate procedures (Liu *et al.*, 2022; Robert, 2009; Imaduddin *et al.*, 2022).

Principle

Celltrac G MEK- g100 uses a helium-neon (He-Ne) laser to measure red blood cells, Platelets, Hematocrit and a tungsten-halogen light beam to measure hemoglobin, white blood cells, and white blood cells differentials, both are focused on the cell stream in the counting chamber of the machine (Robert, 2009). Optical sensors pick up the scattered light impulses at each angle, count and report the number of each cell type counted (Robert, 2009). Like other automated hematology analyzers, Celltrac G MEK calculates other blood cell parameters based on the cell counts (Robert, 2009).

Electrolytes and uric acid analysis

Electrolytes analyser (Selectrapro XL), was used to measure the concentration of electrolytes such as potassium, calcium, phosphate, chlorine and sodium [32]. Uric acid meter (SKU BFI-6369) was used to measure the concentration of uric acid [31].

Principle

The electrolyte analyser uses the principle of ion selective electrodes which measures the voltage that develops between the inner and outer surfaces of an ion selective electrode [32].

Principle

The uric acid analyzer uses enzymatic determination of uric acid which results from the specific oxidation of uric acid by uricase, uricase converts its substrate to allantoin the differential absorbance of these substances at 546 nm allows quantification of uric acid [33].

Reactive proteins (CRP) analysis

C -reactive proteins concentrations in blood was determined using Fincare C – reactive proteins analyzer by photometric measurement at 546 nm or 700 nm of antigen-antibody reaction between antibodies to human CRP bound to polystyrene particles and CRP present in the sample [34].

Statistical analysis

Statistical analysis was done using Statistical Package for Social Science (SPSS, version 24). The results were expressed as Mean $\pm$ Standard Deviation. Multiple group comparisons was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test in order to detect inter-group differences, interpret result and simultaneous comparison of multiple groups. A significant difference was determined when $p < 0.05$, $p < 0.01$ and $p < 0.001$ (represented as letters b, c and d respectively) and no significance $p > 0.05$ (represented as letter a).

Results

Acute toxicity test

The result of the acute toxicity (LD_{50}) of *Manihot esculenta* methanolic extracts in different doses ranging from 1000 mg, 2000 mg and 3000 mg was administered to three groups, group 1, 2 and 3 respectively. Group 1 and 2 mice showed normal behavioural activity and no death was recorded while group 3 mice showed slow behaviour but no death was also recorded. Therefore, the LD_{50} of *Manihot esculenta* methanolic extract is above 3000 mg/kg body weight.

Peripheral blood examination

The result of the peripheral blood smear viewed with 40x magnification showed normal blood cell types in the control group while there was rouleaux formation, blast cells with cuplike morphology in benzene group, groups treated with *Manihot esculenta* methanolic extract showed a better blood cell morphology, absence of rouleaux formation and minimal blast cells. This result aligns with the findings of Ola (2021). Treatment with Dox/Ara C regimen also improved morphology of blood cells and recorded few blast cells.

Biochemical examination

From the biochemical examination of blood we found an increase in lymphocytes and white blood cells level significantly ($p < 0.001$) in benzene group compared to control group. Treatment with *Manihot esculenta* methanolic extract significantly ($p < 0.05$) decreased the lymphocytes and white blood cells level compared to benzene group, in this research there was also a significant ($p < 0.05$) decrease in red blood cells, haemoglobin, mean corpuscular haemoglobin concentration and platelets in benzene group compared to control group. However, supplementation with *Manihot esculenta* methanolic extract resulted in the

restoration ($p < 0.05$) of red blood cells, haemoglobin, platelets and mean corpuscular haemoglobin concentration close to normal ranges (figure 1).

From the data found in figure 2 there was a significant $p < 0.05$ increase in C-reactive proteins, sodium, potassium, chlorine and phosphate while a significant $P < 0.05$ decrease of calcium in benzene group compared to the control which had normal ranges. Treatment with *Manihot esculenta* methanolic extract reversed c-reactive proteins and electrolytes concentration close to normal ranges.

Conversely in figure 3 uric acid concentration increased significantly $p < 0.001$ in benzene group compared to the control. Treatment with *Manihot esculenta* methanolic extract and Dox/Cyt anti-leukemic drug regimen reduced uric acid concentration.

Weight management efficacy of the plant extract

The result in figure 4 below shows a significant ($p < 0.05$) decrease in weight of benzene group compared to the control group. Treatment with *Manihot esculenta* methanolic increased the weight of mice significantly ($p < 0.001$) from day 14 to 21.

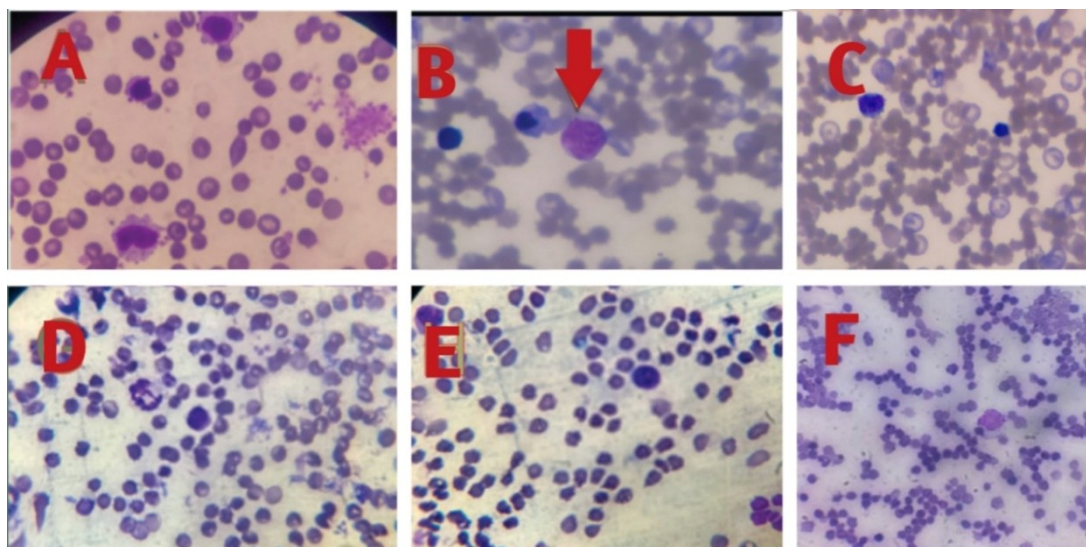


Plate 1: Effect of *Manihot esculenta* methanolic roots extract on peripheral blood microscopy examination. Groups: A= control, B = Benzene (blast cell pointed by red arrow), C= Dox/Ara C, D= 2.5 Mg *Manihot esculenta* methanolic extract, E=5.0 Mg *Manihot esculenta* methanolic extract, F= 10 Mg *Manihot esculenta* methanolic extract.

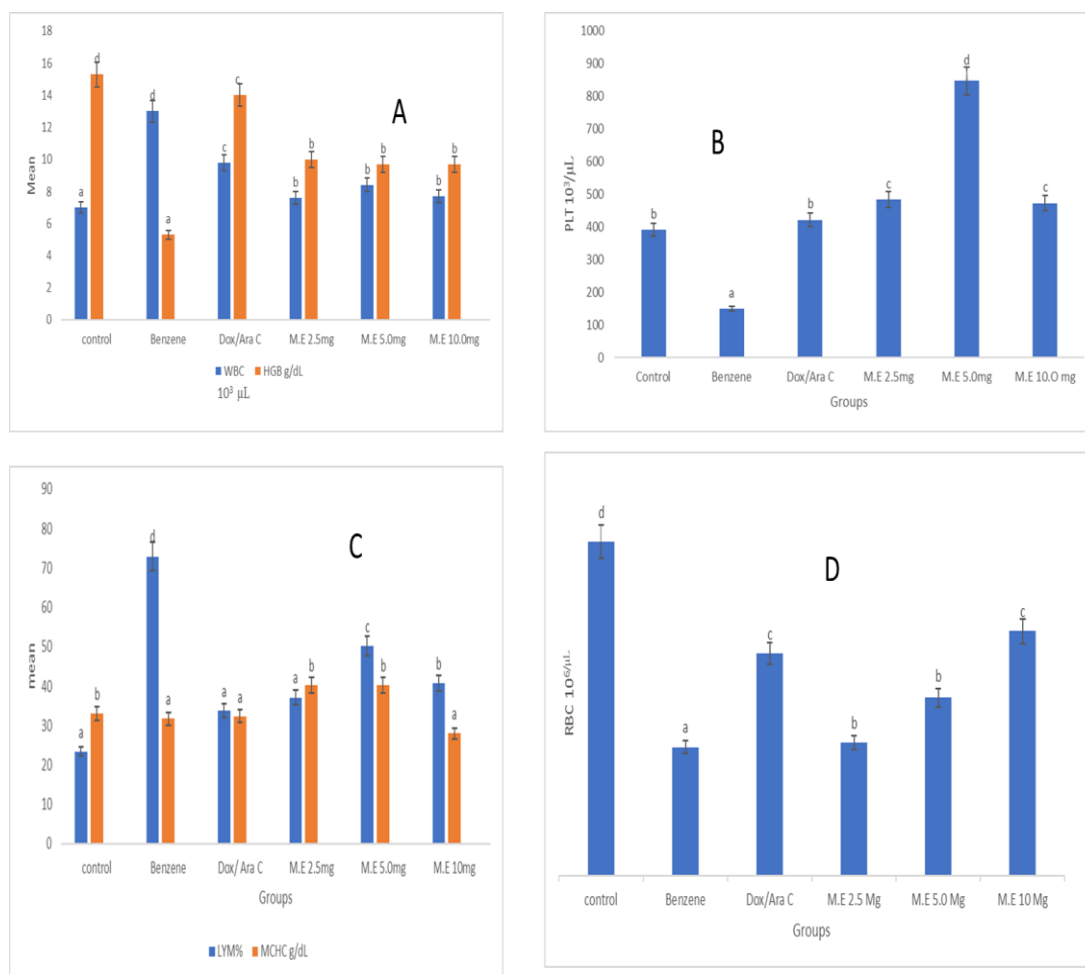


Figure 1: Effect of *Manihot esculenta* methanolic extract on white blood cell/haemoglobin (A), platelets (B), lymphocytes/mean corpuscular haemoglobin (C) and red blood cell (D). Values represented as mean ± standard deviation (n = 5). Bars with different letters differ significantly at p < 0.05.

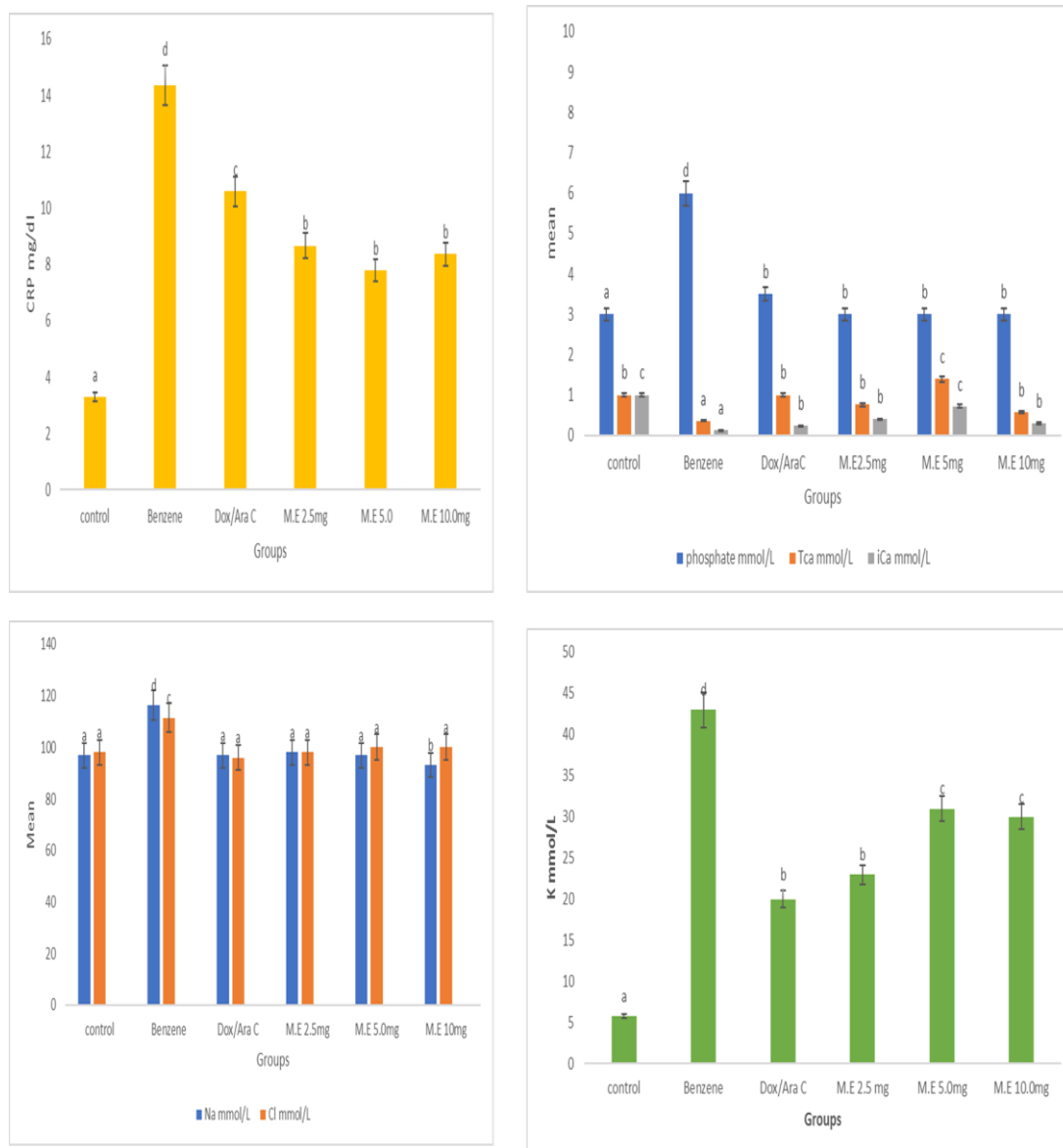


Figure 2: Effect of *Manihot esculenta* methanolic extract on C-reactive protein(A), phosphate/calcium(B), sodium/chlorine (C) and potassium (D). Values represented as mean $\pm$ standard deviation (n = 5). Bars with different letters differ significantly at $p < 0.05$.

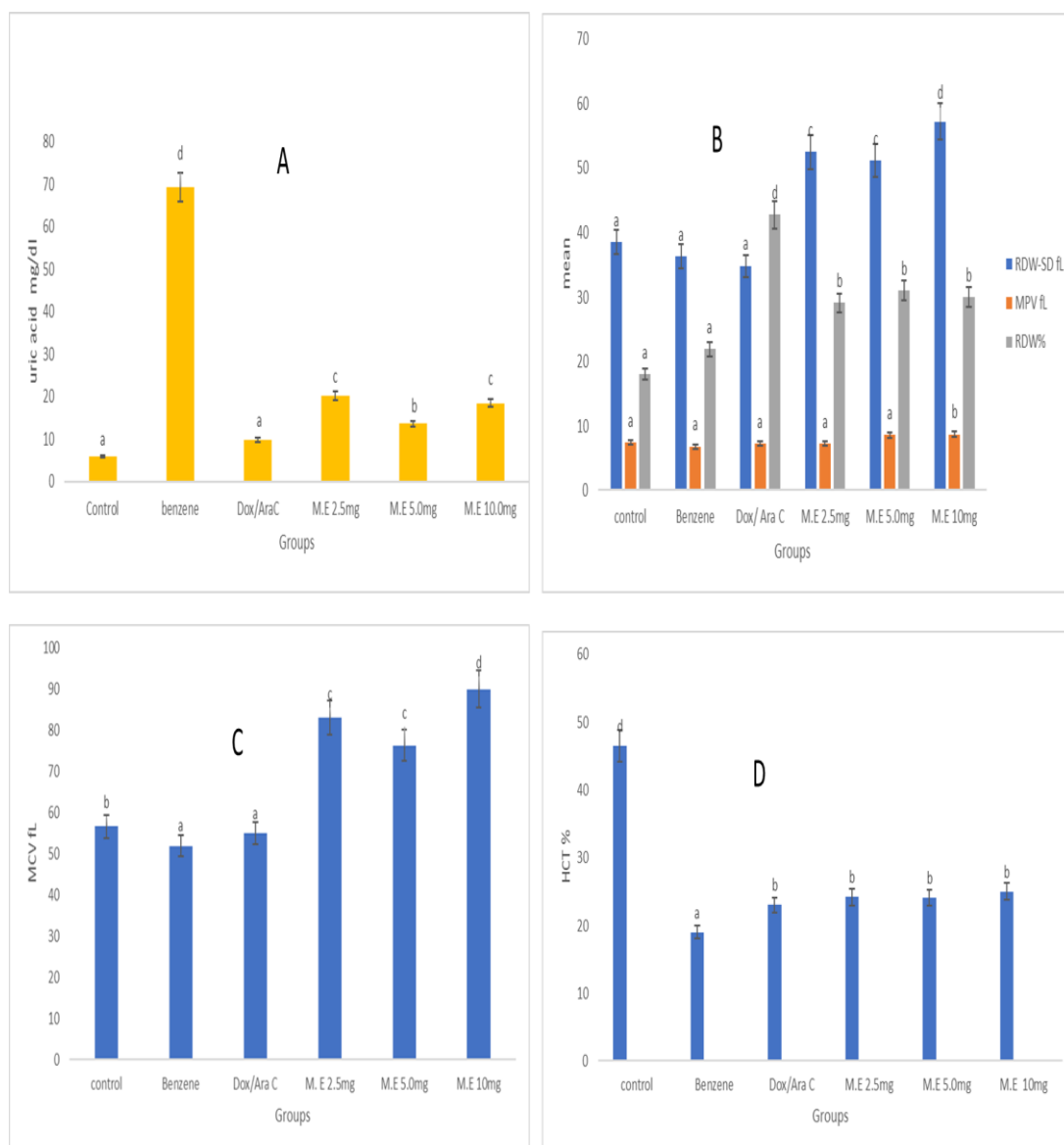


Figure 3: Effect of *Manihot esculenta* methanolic extract on uric acid(A), RDW-SD/MPV/RDW(B), mean corpuscular volume (C) and haematocrit (D). Values represented as mean $\pm$ standard deviation (n = 5). Bars with different letters differ significantly at $p < 0.05$.

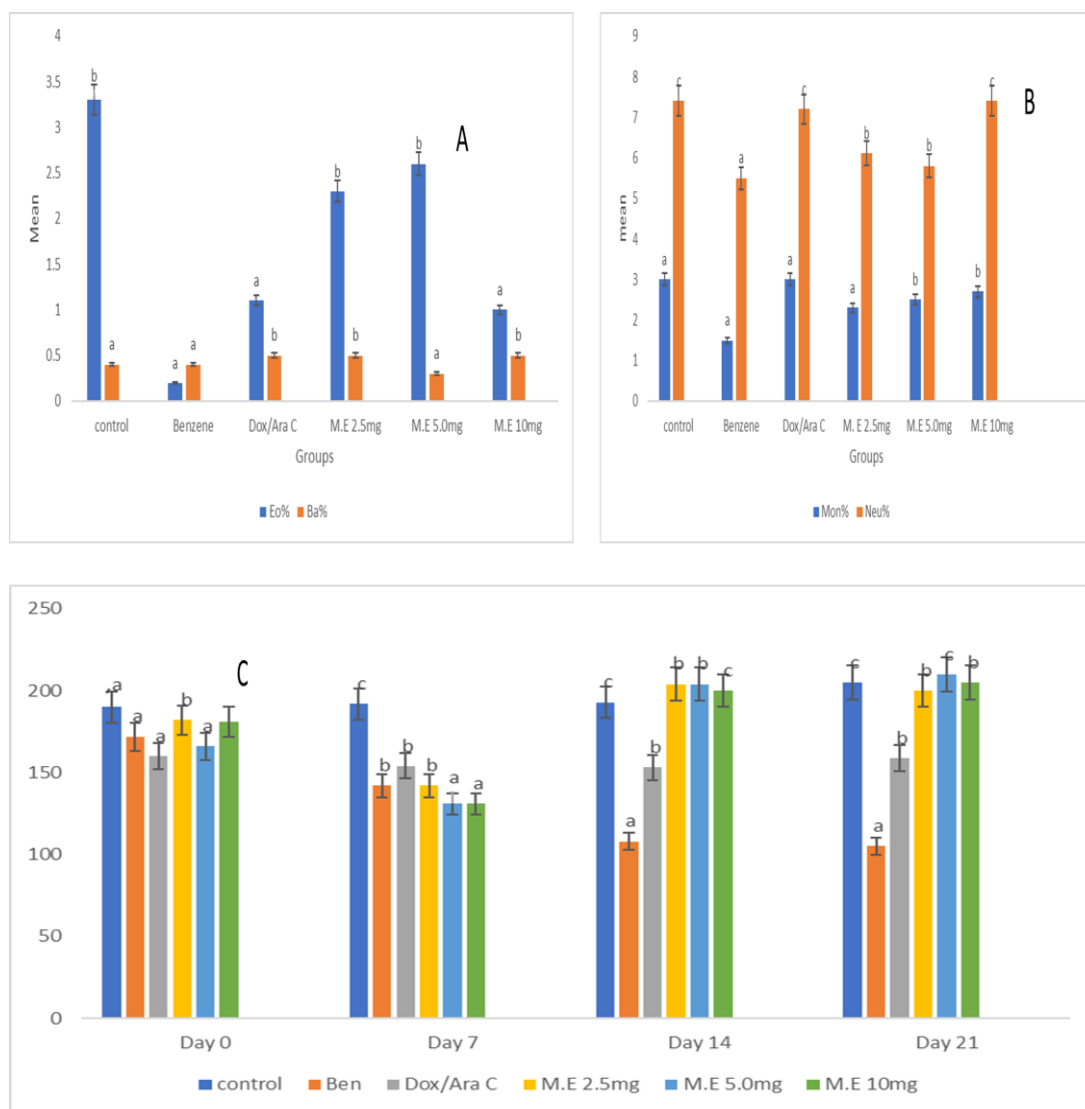


Figure 4: Effect of *Manihot esculenta* methanolic extract on eosinophil and basophils (A), monocytes and neutrophils (B) weight management (C). Values represented as mean $\pm$ standard deviation (n = 5). Bars with different letters differ significantly at $p < 0.05$.

Discussion

Leukemia is a disease of the blood and bone marrow and it is well established that benzene and its metabolites causes chromosomal aberrations in blood and bone marrow of chronically exposed humans and experimental models leading to leukemogenesis (McHale *et al.*, 2012). In this study leukemia was induced successfully in mice using benzene and biochemical evaluation of blood reported elevated white blood cells and reduced red blood cells, haemoglobin and platelets levels significantly $p < 0.05$ in benzene group and treatment with *Manihot esculenta* methanolic extract and Dox/Ara C chemotherapeutic drug regimen reversed haematological parameters close to normal ranges, the results correlate with the findings of Liu *et al.*, 2022; Ola, 2021; Akanni *et al.*, 2017. Leukemia is also characterized by

an increased level of immature leukocytes or blast cells in bone marrow and peripheral blood (Crassini, *et al.*, 2019).

Findings from this research presented leukocytes and blast cells observed in blood microscopy of benzene group and absent in control group, while appropriate groups treated with *Manihot esculenta* methanolic extract had minimal blast cells. This result is similar to the findings of Davi *et al.*, (2022) who reported high concentration of leukocytes in leukemia. Presence of blast cells in blood microscopy further confirms leukemia induction in mice models. Lymphocytosis was observed in benzene group although treatment with *Manihot esculenta* methanolic extract and Dox/Ara C regimen to appropriate groups significantly ($p < 0.05$) decreased lymphocytes level in a dose dependent manner. Mean corpuscular haemoglobin concentration (MCHC) increased significantly ($p < 0.05$) in groups treated with *Manihot esculenta* methanolic extract while there was no significant ($p > 0.05$) difference in MCHC in groups treated with Dox/Ara C regimen and benzene. C- reactive protein is a pentameric protein synthesized by the liver whose level increases in response to inflammation, inflammation is known to play a beneficial role in immune system activation and tissue regeneration, chronic inflammation can lead to haematopoietic stem cell damage resulting to bone marrow failure or development of leukemia, therefore, C-reactive proteins is used to monitor the extent of inflammation in cancer patients (Potempa *et al.*, 2022; Saluja *et al.*, 2024). In this study, C-reactive protein concentration significantly ($p < 0.001$) increased in benzene group and was normal in control group. Supplementation with *Manihot esculenta* methanolic extract after leukemia induction reduced C-reactive proteins concentration significantly ($p < 0.05$). A study by Liang *et al.*, (2023) also recorded high concentrations of c-reactive proteins in leukemia patients which correspond with this result.

The basic electrolytes analyzed in this research were Na^+ , Cl^- , and K^+ which concentrations increased while Ca^{2+} concentration decreased significantly ($p < 0.05$) in benzene group. Treatment with *Manihot esculenta* methanolic extract restored electrolyte concentration close to normal levels. Phosphate levels also increased significantly ($p < 0.001$) in benzene group, supplementation with *Manihot esculenta* methanolic extract decreased phosphate concentration significantly ($p < 0.05$), these results is in tandem with previous research findings of several medicinal plants reported to alleviate hyperkalemia, hyperphosphatemia, and elevate calcium levels close to normal due to the presence of phytochemicals (Imaduddin *et al.*, 2022). Uric acid concentration significantly ($p < 0.001$) increased in benzene group and normal in control group, supplementation with *Manihot esculenta* methanolic extract to appropriate groups stabilized ($p < 0.05$) uric acid concentration close to normal ranges in a dose dependent manner. The hyperuricemia condition recorded in this research aligns with the findings of lai *et al.*, (2020). Treatment with Dox/Ara C regimen also reduced ($p < 0.05$) uric acid levels close to the control group range. There was no significant $p > 0.05$ difference in Red cell distribution width standard deviation concentrations (RCDW-SD) of control, benzene and Dox/ Ara C regimen although RCDW-SD concentrations increased significantly ($p < 0.01$) in groups treated with *Manihot*

esculenta methanolic roots extract in a dose dependent manner. Mean platelet levels (MPL) remain insignificant $p > 0.05$ in all groups. Mean corpuscular volume (MCV) concentration increased significantly $p < 0.05$ in groups treated with *Manihot esculenta* methanolic extract in a dose dependent manner compared to the control, benzene and Dox/Ara C groups. Haematocrit (HCT) concentration significantly ($p < 0.05$) increased in control group and groups treated with *Manihot esculenta* methanolic extract compared to benzene group which had a low HCT concentration. Differential white blood cells eosinophils, basophils, monocytes and neutrophils in figure 4 showed a decreased in concentration ($P < 0.05$) of eosinophils in benzene group compared to control group while there was a significant ($p < 0.01$) increase in eosinophils levels upon treatment with *Manihot esculenta* extract in a dose dependent manner. Eosinophils are innate immune cell which exhibit antiviral, antibacterial and antitumor effector functions and are involved in inflammatory disorders and allergies (Ghaffari and Rezaei, 2023). On the other hand, basophils, neutrophils and monocytes levels also increased significantly $p > 0.05$ in appropriate groups treated with *Manihot esculenta* methanolic extract. Basophils confer anti-tumorigenic activity by releasing pro-inflammatory mediators like tumor necrosis factor alpha (TNF- α) and tryptase (Chauhan *et al.*, 2022). Treatment with *Manihot esculenta* increased the weight of mice significantly ($p < 0.05$) while there was a significant ($p < 0.05$) decrease in weight of mice in benzene group. Increase in body weight is an indication that the plant extract contains nutrients that are properly digested and utilized by the body (Zaruwa *et al.*, 2016). The anti-leukemic and nephro-protective activities of *Manihot esculenta* roots methanolic extract on haematological parameters and kidney function may be attributed to the presence of food nutrients and phytochemicals in the plant. Nutrients such as vitamin C and minerals like iron are reported present in formidable amount in the plant (Kim and Lida, 2023). Iron is needed to maintain cellular homeostasis in haematopoiesis and erythropoiesis (Kim and Lida, 2023). Vitamin C on the other hand acts as a co-factor of TET protein, which acts as a tumor suppressor, promotes TET 2 enzymatic activity and regulates cellular activities such as self-renewal, differentiation and cell survival of haematopoietic stem cell and conversely vitamin C deficiency accelerates leukemogenesis (Mouchel *et al.*, 2023). Furthermore, presence of reported phytochemicals in *Manihot esculenta* such as phenols and flavonoids which has ability to reduce inflammation, scavenge free radicals, enhance DNA damage repair mechanism and cause cytotoxicity in tumor cells also added to the plant performance in mitigating the leukemia in mice ((Kim and Lida, 2023; Yi *et al.*, 2011).

Conclusion

Medicinal plants have demonstrated significant application in the pharmaceutical industry in the production of drugs that combat different diseases, this may be linked to their availability and less toxicity to the overall physiology of humans. *Manihot esculenta* is one of the most edible root vegetables in the world and has been utilized in the food industry for the production of different consumables. This research was designed to evaluate the potentials of *Manihot esculenta* methanolic extract in leukemia. The plant mitigated the

haematological malignancy and kidney disturbance caused by benzene and its metabolites, by significantly $P < 0.05$ maintaining biochemical parameters close to normal ranges. This effect may be attributed to the presence of valuable nutrients and phytochemicals in the plant roots. Therefore, we can conclude that *Manihot esculenta* roots may be used as complementary medicine or prophylaxis in leukemia conditions.

Recommendations

Further studies should be carried out to isolate pure compounds from the plant which can be tested on leukemic cell line and may be used as potential drugs candidates. In-silico studies can also be assessed to evaluate bioactive compounds for druggability and binding affinity with genes or proteins associated with leukemia.

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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

Although our current study focuses on the in-vivo chemo-protective and nephro-protective potentials of *manihot esculenta* roots methanolic extracts. A major limitation was failure to carry out genetic testing such as BCR-ABL1 which is a major biomarker in evaluating the presence and prognosis of leukemia. Other limitations were small experimental size and integration of data into human subjects.

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