International Journal of Research in Medical Sciences
Batubo NP et al. Int J Res Med Sci. 2024 Jan;12(1):61-69
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20233978

Original Research Article

Hematological, histopathological and oxidative stress responses to n-hexane extract of *Terminalia catappa* nuts in leukemia-induced Wistar rats

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Received: 15 November 2023
Revised: 08 December 2023
Accepted: 11 December 2023

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ABSTRACT

Background: *Terminalia catappa* nut possesses antioxidant and anticancer properties, but its effects on leukemia are unclear. This study investigates the effects of n-hexane extract of *Terminalia catappa* nut (TCN) on some hematological parameters, oxidative stress markers, and bone/spleen histopathology in a Wistar rat model of benzene-induced leukemia.
Methods: Leukemia was induced in Wistar rats with 0.2 ml/kg Benzene solution and treated with 200, 400 or 800 mg/kg/day of *Terminalia catappa* nut extract (TCN) for 42 days and with 5-fluorouracil (20 mg/kg) via intraperitoneal injection twice a week for 6 weeks. Hematological parameters, antioxidant markers, and bone and spleen histology were analysed.
Results: All TCN doses significantly lowered elevated WBCs by 32-53% and normalized RBC parameters compared to leukemic controls, mitigating cancer-induced anemia. TCN also exhibited potent antioxidant effects by enhancing SOD, GSH, and catalase while reducing MDA versus untreated rats. Bone marrow analysis revealed TCN conferred dose-dependent benefits on cellularity and architecture, reducing myeloid blasts and leukocyte infiltration. A near-normal bone microarchitecture was attained with the highest TCN dose. Similarly, TCN elicited marked improvements in splenic cytoarchitecture and attenuation of hypercellularity, lymphocytic infiltration and megakaryocytes compared to leukemic controls in a dose-dependent manner.
Conclusions: *Terminalia catappa* nut extract demonstrated anti-leukemic, haematopoietic, antioxidant, and organ protective effects in leukemic Wistar rats induced with benzene solution, supporting its potential as an adjuvant therapeutic agent.

Keywords: Anti-inflammatory, Anti-leukemia, Antioxidant, Hematological parameters, Histopathology of bone and spleen, n-hexane extract, *Terminalia catappa*, Wistar rats

INTRODUCTION

Leukemia is a complex health condition and a significant cause of morbidity and mortality and collectively impacts a substantial portion of the global population.[1,2] Leukemia is a group of malignant disorders of the blood-forming organs, and it is characterized by the uncontrolled proliferation and development of immature white blood cells and their precursors in the bone marrow. Leukemia is the 11th leading cause of cancer-related death globally, with a global prevalence of 32.26% in 2017.[1,2] A recent report from the surveillance, epidemiology, and end results program (SEER) estimates that in 2020, 490,875 people lived with leukemia in the United States. There will be 59,610 (3.0%) new cases of

leukemia and 23,710 (3.9%) cases of death from leukemia in 2023 in the US.[3,4]

The burden of leukemia in Nigeria remains a significant public health concern, as the 2020 WHO Global Cancer Observatory report highlights. With a reported 3,378 new cases, leukemia ranks sixth among all cancers, accounting for 2.7% of the total cancer cases in the country. Leukemia-related mortality is also substantial, with 2,504 recorded deaths, placing it at the identical rank of sixth among cancer-related mortality, translating to a concerning percentage death rate of 3.2%. Notably, leukemia exhibits a higher prevalence in males, with a gender ratio of 1:1.2, underscoring the need for comprehensive strategies to address the disparities in disease occurrence and outcomes in Nigeria.[5]

Despite being a malignancy primarily affecting the blood-forming organs, Leukemia exerts profound systemic effects on diverse physiological processes, including metabolism. Despite anti-leukemic treatment, survivors of childhood leukemia remain susceptible to an elevated metabolic syndrome risk.[6,7] The prevalence of metabolic syndrome remains higher in children, and this risk remains pronounced throughout the treatment and post-treatment phases.[7] Chemotherapy and radiation therapy are the primary treatment options for leukemia; however, their efficacy is frequently hampered by significant adverse effects.[8]

Consequently, there is a growing demand for alternative therapeutic approaches that offer minimal side effects. Nutritional support has shown promising benefits in improving nutritional status, reducing complications, and enhancing hematological outcomes.[9,10] Natural products have long been utilised in traditional medicine systems and have drawn attention for their diverse pharmacological activities.[11,12]

Terminalia catappa, commonly known as the Indian almond or tropical almond, is a tropical tree belonging to the Combretaceae family. Various parts of this plant, including its nut, leaves, and bark, have been traditionally used in folk medicine for their purported health benefits.[13] *Terminalia catappa* nut extract is believed to possess antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, making it a potential plant for exploration in leukemia management.[14,15] Several studies reported that *Terminalia catappa* fruit extract resulted in increased haemoglobin (Hb) and red blood cell (RBC) levels while decreasing white blood cell (WBC), platelet count, and erythrocyte sedimentation rate (ESR).[16,17]

Given the diverse medicinal properties of *Terminalia catappa* and growing interest in exploring its therapeutic potential, the role of *Terminalia catappa* nuts in leukemia is yet to be explored. Therefore, investigating the effect of *Terminalia catappa* nuts on leukemia could hold promising therapeutic implications. Therefore, this study aimed to investigate the metabolic response to n-hexane extract of *Terminalia catappa* nuts on hematological, oxidative stress parameters and histopathology of bone and spleen in Wistar rats induced with leukemia. By elucidating the potential benefits, this study aims to shed light on the nutritional potential of *Terminalia catappa* nuts as a possible supportive intervention for individuals with leukemia and a complementary treatment option for leukemia.

METHODS

Study design

This study was an experimentally controlled study in 70 male Wistar rats to evaluate the efficacy of n-hexane extract of *Terminalia catappa* nut (TCN) on benzene-induced leukemia, which was induced by intravenous benzene injection for 6 weeks. The study was conducted from August 2023 to October 2023 at the animal house of the department of human physiology, University of Port Harcourt, Nigeria.

Ethical consideration

The animal experiments were performed per the Institute of Laboratory Animal Resources guidelines, a guide for the care and use of laboratory animals.[18] The study protocol was reviewed and approved by the research ethics committee on 10 August 2023 with approval number UPH/CERMAD/REC/MM90/216.

Collection, identification, and preparation of plants

The fresh fruits of *Terminalia catappa* were obtained between August and November 2022 from the University of Port Harcourt and Rivers State University, Port Harcourt, Nigeria. The fruits were cleaned under running water immediately after collection, drained, and pat-dried off. The edible nut (nut) of *Terminalia catappa* was removed from the fibrous husk and the hard shell. The processed nuts were air-dried at room temperature. Once dried, the nuts were finely ground into a homogenous powder and extracted with 85% aqueous n-hexane using a solid-to-liquid ratio of 1:30 (w/v).[19] The extraction procedure was repeated four times to ensure that the extraction was adequately exhaustive, and the solvent extract was collected for each batch. The collected extracts of *Terminalia catappa* nuts were pooled, filtered, and concentrated under vacuum, using a rotary evaporator (Heidolph GmbH and Co. K. G., Germany), freeze-dried and kept at -20°C before administration.

Experimental animals

The study employed a controlled experimental design using male Wistar rats as the animal model. 70 four- to six-week-old male Wistar rats weighing between 80-100 gm were procured from the Rivers State University Animal House Port Harcourt, Nigeria. The rats were housed in compartmentalized cages and provided a two-

week acclimatization period under a controlled daily light and dark cycle of 12 hours. The rats had unrestricted access to standard feed and clean drinking water *ad libitum.*

Experimental design

Preparation of benzene solution

Benzene solution (product of Sigma-Aldrich with Cat number 270709 and >99.9%) and 2-propanol (product of Sigma-Aldrich with Cat number 270709 and >99.9%) were obtained from the chemistry department at the Rivers State University, Nigeria. The benzene solution was prepared by a mixture of 1 gm/ml of benzene and 2-propanol in a ratio of 1:10 and diluted in distilled water (1 gm/ml) and administered at 1 ml/kg body weight (BW) using the methods of Olufemi et al.[20]

Study protocol

Induction of leukemia

Leukemia was induced in all rats except the negative (normal) control group. Sixty (n=60) male Wistar rats were initially used to induce leukemia for 42 days. Before the induction, baseline hematological parameters (white blood cell count, neutrophil count, lymphocyte count, and haemoglobin level) were assessed by collecting blood samples from the tail veins of the rats. The method of Olufemi et al was used to induce leukemia in Wister rats by the intravenous administration of benzene solution (0.2 ml/kg body weight) every two days for six consecutive weeks through the tail veins of the rats.[20] After six weeks of induction, blood samples were collected from the rats' tail veins for laboratory confirmation (white blood cell count, neutrophil count, lymphocyte count, and hemoglobin level) of leukemia induction in the Wistar rats. Wister rats with elevated white blood cell count, neutrophil count, lymphocyte count, and decreased hemoglobin post-induction were selected and randomly divided into 5 (n=10) groups (Figure 1).

Administration of Terminalia catappa nut extract

The negative control (*n*=10) comprised non-leukemia-induced male Wistar rats, and they were provided with water and fed with standard rat pellets *ad libitum* throughout the experimental period. Leukemia control (n=10) encompassed rats induced with leukemia without administering n-hexane extract of *Terminalia catappa* nuts (TCN). This group of Wistar rats were also provided with water and fed with standard rat pellets *ad libitum* throughout the experimental period. Groups A, B, and C were composed of leukemic-induced Wistar rats and were treated with 200 mg/kg, 400 mg/kg and 800 mg/kg of TCN extract, administered in 2 ml, respectively. Group D, comprising leukaemic-induced Wistar rats, was treated with 5-fluorouracil (20 mg/kg) via intraperitoneal injection twice a week for 6 weeks. The *Terminalia catappa* nut (TCN) extracts were administered daily by oral gavage for 42 days, starting 24 hours after laboratory confirmation of leukemia (Figure 1). All rats received distilled water and standard rat pellets *ad libitum* throughout the experimental period.

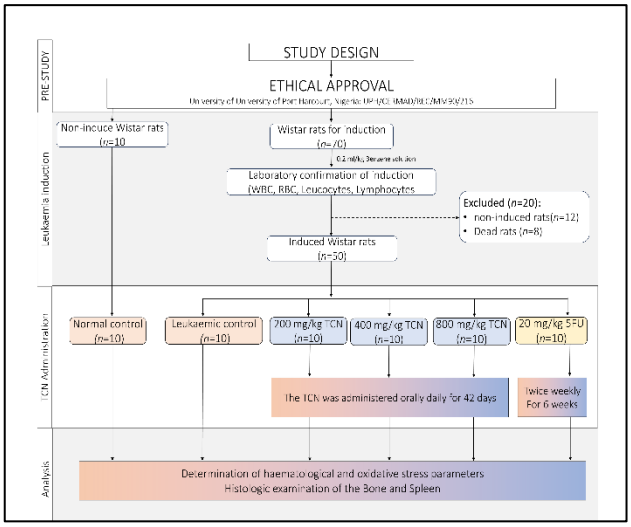

Figure 1: Study protocol and administration of *Terminalia catappa* nut extract (TCN) and 5-fluorouracil (5FU).

Sampling collection

At the end of the experimental period of 42 days, the rats were fasted overnight. The six (6) Wistar rats were anaesthetized with chloroform. Blood samples were collected from the rats through the cardiac puncture into K_2EDTA (ethylenediaminetetraacetic acid) and plain bottles for the determination of hematological parameters, such as red blood cell count, hemoglobin content, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration and oxidative stress markers, were assessed using standard laboratory methods. The bone and spleen were harvested for histological examination.

Hematological determination

Red blood cell (RBC) count, hemoglobin content (HGB), mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count, and differential leukocyte count of white blood cell (neutrophil and lymphocyte) were determined using an automated hematology analyser (Mindray Automated analyser Sysmex XN-550) following the manufactural instructions.

Oxidative stress markers determination

Superoxide dismutase (SOD) activity was measured using the kinetic protocol described by McCord and Fridovich.[21] This assay quantifies SOD enzymatic

activity based on inhibition of superoxide-dependent cytochrome c reduction. Malondialdehyde (MDA) and reduced glutathione (GSH) and peroxidase (GPx) levels were determined spectrophotometrically using the thiobarbituric acid reacting substance (TBARS) assay and Ellman's reagent, respectively.[22,23] The TBARS assay measures lipid peroxidation through the MDA and thiobarbituric acid reaction. Ellman's reagent allows colourimetric assessment of glutathione concentration. Catalase (CAT) activity was evaluated by measuring the enzymatic decomposition of hydrogen peroxide as the substrate based on the protocol by Beers and Sizer.[24]

Histopathological examination

The bone and spleen tissue samples were fixed in neutral buffered formalin, processed, and embedded in paraffin wax. Thin sections of 5 µm thickness were cut using a rotary microtome and placed on glass slides. The sections were then deparaffinised, rehydrated and stained with haematoxylin and eosin (H and E), as described by Fischer et al.[25] The stained sections were examined by light microscopy under ×400 magnification to assess tissue architecture and identify any morphological abnormalities compared to normal histology.

Statistical analysis

The hematological and oxidative stress data results were analysed using a one-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc analysis. The results were expressed as mean±standard error of the mean (SEM). A p value <0.05 was considered statistically significant. All analyses were performed using R statistical software (v 4.3.1).[26]

RESULTS

Effect of Terminalia catappa nut on WBC and differentials

Induction of leukemia significantly increased WBC counts to $17.3\pm0.01\times10^9$ cells/l compared to negative controls at $7.49\pm0.01\times10^9$ cells/l ($p<0.05$) (Table 1). TCN extract at all doses (200, 400, 800 mg/kg) significantly lowered elevated WBC counts by 32-53% ($p<0.05$) (Table 1).

Similarly, untreated leukemic rats showed increased neutrophil counts of $6.60\pm0.03\times10^9$ cells/l versus $1.71\pm0.03\times10^9$ cells/l in negative controls ($p<0.05$). TCN treatment dose-dependently reduced neutrophils by 67-76% ($p<0.05$). Lymphocyte counts were elevated in leukemic controls at $13.2\pm0.56\times10^9$ cells/l compared to the normal controls at $5.70\pm0.01\times10^9$ cells/l ($p<0.05$). Although the lymphocyte counts remained higher than the negative control, lymphocyte counts were reduced by 22-43% by TCN treatment ($p<0.05$) (Table 1).

Effect of Terminalia catappa nut on RBC and indices

Leukemic rats showed decreased RBC count ($6.34\pm0.03\times10^{12}$ cells/l), haemoglobin (12.7±0.10 gm/dl) and hematocrit (47.8±0.27%) compared to negative controls ($p<0.05$), indicating anemia (Table 1). TCN dose-dependently increased RBC count by 10-20%, hemoglobin by 2-11%, hematocrit by 1-5%, and normalized MCV, MCH and MCHC compared to untreated leukemic rats ($p<0.05$) (Table 1). The high-dose TCN demonstrated greater efficacy than 5-fluorouracil (Table 1).

Table 1: Effect of *Terminalia catappa* nut (TCN) extract treatment on some hematological parameters (n=6) of benzene-induced leukemic Wistar rats.

Parameters	Negative control	Leukaemic control	200 mg/kg TCN	400 mg/kg TCN	800 mg/kg TCN	20 mg/kg 5FU
WBC (10^9 cells/l)	7.49±0.01	17.3±0.01*	11.6±0.24$^{*\#a}$	1.83±0.11$^{*\#a}$	1.61±0.31$^{*\#a}$	7.73±0.07$^{\#}$
NEU (10^9 cells/l)	1.71±0.03	6.60±0.03*	2.17±0.04$^{*\#}$	1.83±0.03$^{\#}$	1.61±0.01$^{\#}$	1.78±0.01$^{\#}$
LYM (10^9 cells/l)	5.70±0.01	13.2±0.56*	10.3±0.23$^{*\#}$	7.55±0.04$^{*\#}$	7.58±0.31$^{*\#}$	6.53±0.17
Platelet (10^9 cells/l)	731±0.49	878±0.55*	781±0.40$^{*\#}$	736±0.40$^{*\#}$	687±0.40$^{*\#}$	689±0.40*
RBC (10^{12} cells/l)	7.18±0.01	6.34±0.03*	6.98±0.08$^{\#}$	7.13±0.04$^{\#a}$	7.59±0.05$^{*\#a}$	6.79±0.01
HB (gm/dl)	13.6±0.02	12.7±0.10*	12.4±0.08*	13.3±0.01$^{\#}$	14.1±0.26$^{\#a}$	12.8±0.16*
MCV (fl)	51.4±0.05	47.8±0.27*	47.6±0.06^{*a}	49.1±0.10$^{*\#}$	50.2±0.58$^{*\#}$	49.4±0.04$^{*\#}$
MCH (pg)	19.1±0.03	17.1±0.06*	17.8±0.06*	18.6±0.10$^{*\#a}$	18.9±0.58$^{\#a}$	18.2±0.05$^{*\#}$
MCHC (gm/l)	377±0.40	367±1.20*	376±0.25$^{\#a}$	377±0.60$^{\#a}$	381±0.20$^{*\#a}$	372±0.20$^{*\#}$

Data are presented as mean±standard error of the mean, *p<0.05 when compared with the negative control, #p<0.05, was significant compared with leukaemic control. At the same time, ap < 0.05 was significant when compared with the 20 mg/kg 5FU. WBC: white blood cell count; NEU: neutrophil count; LYM: lymphocyte count; RBC: red blood cells, HB: hemoglobin, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, 5FU: 5-fluorouracil.

Table 2: Effect of *Terminalia catappa* nut (TCN) extract treatment on some oxidative stress parameters (n=6) of benzene-induced leukemic Wistar rats.

Parameters	Negative control	Leukaemic control	200 mg/kg TCN	400 mg/kg TCN	800 mg/kg TCN	20 mg/kg 5FU
SOD (U/mg protein)	1.64±0.01	0.45±0.01*	1.35±0.01$^{*\#a}$	1.42±0.01*#a	1.53±0.01$^{*\#a}$	1.38±0.01$^{*\#}$
GSH (μM/mg protein)	7.81±0.01	2.41±0.01*	3.31±0.01$^{*\#a}$	6.76±0.01*#a	6.74±0.01$^{*\#a}$	4.76±0.01$^{*\#}$
CAT (U/mg protein)	12.0±0.01	6.74±0.01*	11.8±0.02$^{*\#}$	13.0±0.04*#	9.61±0.01$^{*\#}$	12.1±0.01$^{\#}$
MDA (ηM/mg protein)	1.20±0.01	2.17±0.01*	1.88±0.01$^{*\#a}$	1.42±0.01*#a	1.36±0.01$^{*\#a}$	1.66±0.01$^{*\#}$
GPx (U/mg protein)	3.5±0.02	1.2±0.001*	2.1±0.01$^{*\#}$	2.8±0.02*#	3.0±0.03$^{*\#}$	2.5±0.01$^{*\#}$

All values are expressed as Mean±standard error of the mean, (n=6), *p<0.05 when compared with the negative control, $^{\#}$p<0.05, was significant compared with leukemic control. At the same time, ^{a}p<0.05 was significant when compared with the 20 mg/kg 5FU. SOD: superoxide dismutase, GSH: glutathione; GPx: glutathione peroxidase, CAT: catalase; MDA: malondialdehyde, 5FU: 5-flourouracil.

Effect of Terminalia catappa nut platelet counts

Platelet counts were elevated in leukemic control rats (878±0.55×10^{9} cells/l) compared to negative controls (731±0.49×10^{9} cells/l) (p<0.05). TCN treatment at 200, 400 and 800 mg/kg significantly lowered platelet counts by 11%, 16% and 22%, respectively, compared to leukemic controls (p<0.05) (Table 1).

Effect of Terminalia catappa nut on oxidative stress markers

Leukemic Wistar rats exhibited lowered SOD (0.45±0.01 U/mg protein), GSH (2.41±0.01 μM/mg protein), GPx (1.2±0.001 U/mg protein) and catalase (6.74±0.01 U/mg protein) along with elevated MDA (2.17±0.01 nM/mg protein) compared negative controls (p<0.05), indicating oxidative stress (Table 2). TCN dose-dependently increased SOD by 200-233%, GSH by 38-179%, catalase by 76-93%, and decreased MDA by 13-30% compared to leukemic controls (p<0.05) (Table 2). The high dose TCN showed superior antioxidant effects over 5-fluorouracil (Table 2).

Effect of Terminalia catappa nut on organ pathology

Histopathology of the bone

The histological examination of the bones showed distinct differences across the treatment groups. The negative control (Figure 2A) of the femur metaphysis displayed normal bone marrow cellularity and microarchitecture with abundant osteocytes, no blast cells, or leukemia infiltrates. In contrast, the leukemic control (Figure 2B) of the femur diaphysis exhibited marked hypercellularity due to diffuse infiltration by immature myeloid blasts indicative of acute myeloid leukemia (AML). There was disruption of the normal architecture throughout the imaged area.

The 200 mg/kg TCN (Figure 2C) tibia section showed moderate hypercellularity, some myeloid blasts, mild microarchitectural disruption, and a small, clustered AML infiltrate. The 400 mg/kg TCN femur metaphysis (Figure 2D) appeared mildly hypercellular with rare blasts, improved architecture, and a minor AML infiltrate. The 800 mg/kg TCN femur diaphysis (Figure 2E) resembled the negative control with normal cellularity, no blasts, intact architecture, and abundant osteocytes. Finally, the 20 mg/kg 5FU tibia diaphysis (Figure 2F) exhibited hypocellular marrow without blasts but disrupted architecture and dense AML infiltrates.

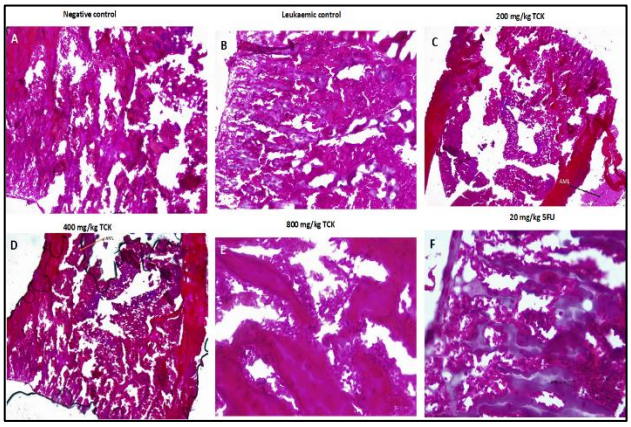

Figure 2: Photomicrograph of bone section (H and E, ×400) from experimental rats after 42 days of treatment with n-hexane extract of *Terminalia catappa* nuts: A) Negative control, B) leukaemic control, C) 200 mg/kg TCN group, D) 400 mg/kg TCN group, E) 800 mg/kg TCN group, F) 20 mg/kg 5FU group.
TCN: *Terminalia catappa* nut extract, 5FU: 5-fluorouracil.

Histopathology of the spleen

The histological examination of the spleen showed distinct differences between the treatment groups. The negative control (Figure 3A) displayed normal splenic architecture with distinct red and white pulp containing lymphocytes, with no signs of cellular activation. In contrast, the leukemic control (Figure 3B) exhibited massive infiltration and destruction of the splenic architecture by immature myeloid blasts, along with lymphoid depletion, necrosis, megakaryocyte infiltration and capsular abnormalities indicative of leukemia progression.

The 200 mg/kg TCN (Figure 3C) showed some improvement with increased T-cells and reduced megakaryocytes, mast cells and erythroid cells. The 400 mg/kg TCN (Figure 3D) demonstrated further benefits with less lymphocytic infiltration, while the 800 mg/kg TCN (Figure 3E) displayed near-normal splenic histoarchitecture approaching that of the negative control. The 5FU group (Figure 3F) also showed some reduction in leukemic cells and improved white pulp morphology compared to the leukemic control, although not to the same extent as the highest TCN dose.

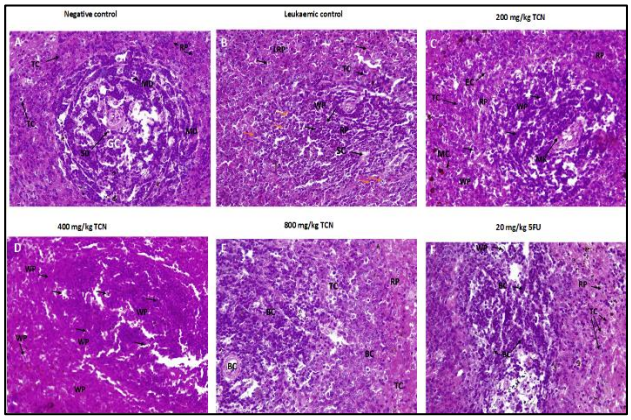

Figure 3: Photomicrograph of spleen section (H and E, x400) from experimental rats after 42 days of treatment with n-hexane extract of *Terminalia catappa* nuts: A) negative control, B) leukaemic control, C) 200 mg/kg TCN group, D) 400 mg/kg TCN group, E) 800 mg/kg TCN group, F) 20 mg/kg 5FU group.

RP: Red pulp, WP: White pulp, TC: T lymphocytes, BC: B lymphocytes, SD: Small dense lymphocytes, MD: Medium dense lymphocytes, MC: Mast cells, Nodular dense infiltration of myeloid cells (Black arrows), Necrosis (MFN) around splenic arterioles, LWP: splenic white pulp (LWP): Red arrows: Megakaryocyte infiltration with erythroid precursors. TCN: Terminalia catappa nut extract, 5FU: 5-fluorouracil.

DISCUSSION

This study investigated the effects of *n*-hexane extract of *Terminalia catappa* nut (TCN) on some hematological, antioxidant, and histopathological effects of bone and spleen in Wistar rats induced with leukemia. This study demonstrated the protective effects of n-hexane extract *Terminalia catappa* nut against hematological, oxidative stress and histopathology of the bone and spleen alterations in Wistar rats of benzene-induced leukemia. TCN ameliorated leukocytosis, anaemia, thrombocytosis, and oxidative stress in a dose-dependent manner.

Induction of leukemia resulted in significant leukocytosis, neutrophilia, lymphocytosis, and thrombocytosis, confirming the presence of hematological malignancy and bone marrow hyperplasia, consistent with previous studies.[27-29] Administration of *Terminalia catappa* nut extract (TCN) dose-dependently reduced elevated white blood cell counts and differentials, likely by inhibiting rapid cancer cell proliferation. However, lymphocyte counts remained above normal levels, suggesting higher TCN doses are needed to resolve leukemic lymphocytosis fully.

Previous studies have explored the immunomodulatory effects of *Terminalia catappa* extract.

Chika et al conducted a similar study and found that the leaf extract exhibited anti-inflammatory properties and reduced white blood cell count in an albino rat inflammation model.[30]

Another study conducted by Behl et al. reported similar findings of decreased white blood cell count in rats treated with the nut extract, indicating its potential immune-regulatory effects.[16] Untreated leukemic rats developed significant anemia, as indicated by the reduced red blood cell parameters. TCN treatment not only prevented the worsening of anemia but also dose-dependently improved red blood cell count, hemoglobin, hematocrit, and normalized red blood cell indices such as mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. These erythropoietic effects suggest that TCN may stimulate erythrocyte formation and maturation while protecting against leukemic hematopoiesis and bone marrow suppression.

Furthermore, TCN also lowered elevated platelet counts, thereby reducing thrombocytosis secondary to leukemia and increased erythrocytes, hematocrit, and hemoglobin levels in Wistar rats.[17,30] While these studies do not directly focus on the nut extract but rather the leaf extract of *Terminalia catappa* on red blood cell count, they suggest a potential influence of the extract on hematological parameters. Pandya et al. conducted a study to evaluate the antitumor and antioxidant properties of ethanol extract from *Terminalia catappa* leaves against Ehrlich ascites carcinoma (EAC) in Swiss albino mice, reporting a decrease in white blood cell count and differentials upon treatment with *Terminalia catappa* leaves extract.[31] Our study observed the improvements in anaemia are in line with the presence of folate, iron, vitamins, and phytochemicals in TCN, which are known to stimulate erythropoiesis.[17,32] The observed increase in red blood cell counts and hemoglobin levels indicates the potential of *Terminalia catappa* nut extract as a hematopoietic agent, offering the possibility of enhancing red blood cell production and hemoglobin levels in Wistar rats induced with leukemia.

Excessive production of reactive oxygen species and diminished antioxidant defenses result in oxidative stress, exacerbating hematological cancers.[33,34] A promising therapeutic approach involves restoring redox balance. *Terminalia catappa* nut extract (TCN) demonstrated a dose-dependent elevation in the activity of superoxide dismutase (SOD), glutathione (GSH), and catalase,

accompanied by a reduction in elevated lipid peroxidation. These findings align with earlier reports of crude TCN extracts demonstrating anti-proliferative, pro-apoptotic, and antioxidant properties in various cancer cell lines. These effects have been attributed to bioactive constituents such as gallic acid, flavonoids, tannins, saponins, and triterpenes.[35,36] This suggests that the n-hexane extract used in the present study contains more potent bioactive fractions. This underscores the potent in vivo antioxidant and anti-lipid peroxidative effects of TCN against oxidative damage in cases of leukemia.

The histological improvements in the bone marrow and spleen also demonstrate the dose-dependent anti-leukemic potential of TCN. The attenuation of architectural disruption, reduced blast infiltration, and enhanced lymphocyte populations with TCN treatment concur with prior studies showing *T. catappa* leaf and seed extracts mitigate bone marrow suppression and improve hematopoiesis in myelosuppressed animal models.[37] The dose-dependent benefits indicate that TCN holds promise as an adjuvant therapy for hematological malignancies. *Terminalia catappa* contains bioactive phytochemicals like tannins, triterpenoids, phytosterols, and flavonoids that likely mediate anti-leukemic effects through antioxidant, immunomodulatory and hematopoietic mechanisms based on earlier research.[38]

Clinical and nutritional significance

The antioxidant, anti-anaemic, and organ-protective effects suggest *Terminalia catappa* nut (TCN) may mitigate chemotherapy-induced side effects like oxidative damage and myelosuppression in leukemia patients.[39] TCN could synergise with and enhance current leukemia treatment regimens to sensitize cancer cells, mitigate side effects and enhance treatment outcomes. The results also support the nutritional benefits of *Terminalia catappa* nuts against diseases involving oxidative stress. However, rigorous clinical trials are first needed to evaluate safety and efficacy in human subjects. Our results add to preclinical evidence supporting the traditional medicinal uses of TCN.

A key limitation of this preliminary study is the need for more investigation into the mechanisms and bioactive compounds mediating the hematological and antioxidant effects observed. Testing higher TCN doses would also help further determine optimal concentrations and characterized dose-response relationships. Strengths include the robust leukemia model, dose-dependent study design, and battery of assessed hematological and oxidative stress parameters. Findings were strengthened by quantitative reporting of effects versus controls.

CONCLUSION

This study provides evidence that *Terminalia catappa* nut extract demonstrated protective hematological and antioxidant effects, mitigates leukemic disruption of bone marrow and spleen architecture in a dose-related manner and holds promise as a nutritional adjunct for reducing leukemic hematological complications and oxidative stress.

ACKNOWLEDGEMENTS

We thank Drs. Austin Ajah, Kemzi Elechi-Amadi, Imaobong Nonju, Edith Reuben, Okpara Elile Peace and Elvis Godom for providing technical assistance.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the University of Port Harcourt ethics committee on 10th August 2023 with approval number UPH/CERMAD/REC/MM90/216

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Cite this article as: Batubo NP, Ogbu OS, Victor DD. Hematological, histopathological and oxidative stress responses to n-hexane extract of *Terminalia catappa* nuts in leukemia-induced Wistar rats. Int J Res Med Sci 2024;12:61-9.