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## ***Kigelia africana* Stem Bark, Fruit and Leaf Extracts Alleviate Benzene-induced Leukaemia in Rats**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors OIO and AEO designed and coordinated the study. Authors ABD and IM performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OA, SB and AT managed the analyses of the study. Authors AEO, OIO and ABD assessed the participants clinically for signs of inflammation. Author OA coordinated sample collection while authors SB and AT managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** This study was aimed at evaluating the potential leukaemia chemotherapeutic activities of various extracts of *Kigelia africana* stem bark; fruit and leaf by using a benzene-induced model of leukaemia to validate its folkloric use. Evidences from African herbal medicine and traditional applications have shown that *Kigelia africana* plant has several beneficial therapeutic properties against microbial infections and cancer cell lines.

**Study Design:** Experimental study.

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**Place and Duration of Study:** Department of Biomedical Science, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria and study was conducted between August, 2016 and November, 2016.

**Methodology:** Rats were administered 0.2 mL of benzene solution intravenously through the tail 48-hourly for 4 weeks, tested periodically and observed till leukaemia developed prior to treatment with respective extract of the *Kigelia africana* stem bark, fruit and leaf in appropriate rat groups after leukaemia was confirmed with haematological protocols. Leukaemic rats were administered with 100 mg/mL contained in 0.5 mL stem bark, 0.5 mL fruit and 0.2 mL of the leaf extract orally by gavage using oral cannula once daily post leukemia induction for four weeks. Haematological parameters and white blood cells differential counts (lymphocytes) were assessed in both control and treatment groups to determine the leukaemia burden.

**Results:** *Kigelia africana* treatment using the stem bark, fruit and leaf significantly ( $P < 0.05$ ) alleviated the anaemia indices and reduced the marked leucocytosis usually associated with leukaemia toward the negative control level when compared with the leukaemia control group. Antileukaemic activity however appears to be highest in stem bark, and least in the leaf.

**Conclusion:** This study revealed the potential of ethanol extracts of *Kigelia africana* stem bark, fruit and leaf to reverse leukaemic effects in benzene-induced leukaemia bearing wistar rats and this suggest that the extracts might be promising natural, non-toxic and anticancer agents.

**Keywords:** *Kigelia africana*; benzene; cancer; chemotherapy; leukaemia; chemoprevention.

## 1. INTRODUCTION

The incidence of deadly diseases globally is alarming, which has geared a number of scientists into research and experiment on various causes of these diseases. One of the deadliest diseases currently ravaging and killing humans and even animals is cancer [1]. Cancer is a broad group of various diseases involving unregulated abnormal cells growth anywhere in the body and develops in almost any organ or tissue and it occurs in many forms of over 200 different types [1]. Leukaemia has been described as a cancer of the blood or bone marrow affecting haemopoietic stem cell compartment, characterised by uncontrolled proliferation and accumulation of malignant leukocytes in the bone marrow and peripheral blood usually results from somatic mutations in the DNA [2]. Some mutations trigger this haematological malignancy by activating oncogenes or deactivating tumour suppressor genes consequently disrupting the regulation of cell death or differentiation. These mutations found even within the African continent and beyond, may occur spontaneously or could result from exposure to radiation or carcinogens such as benzene [3].

Cancer treatment with the aid of chemotherapy and radiotherapy have not been fully maximized in patients because of their numerous severe side-effects and toxicity, while the use of several anticancer agents derived from plants are being employed in clinical use all around the globe without harming the normal cells of the body [4,5]. Herbal medicines play a vital role in the

prevention and treatment of cancer and are commonly available and comparatively economical. Many of these medicinal herbs are presently being investigated for novel drugs or templates for the development of new therapeutic agents [6,7].

*Kigelia africana* (Lam.) Benth is a tropical African plant found on riverbanks, where it may reach 20m, along streams and on flood plains, also in open woodland, from KwaZulu-Natal to Tanzania [8]. The plant is widely grown and distributed in South, Central and West Africa belonging to the family of *Bignoniaceae* and commonly called the "Sausage tree" because of its huge fruits. The African common names include the 'pandoro' (Yoruba), 'uturubien' (Ibo) and 'Hantsar giwaa' (Hausa) [9]. It is widely grown as an ornamental plant in tropical regions for its decorative flowers and used throughout Africa, India and the Middle East whereby the tree has been widely cultivated as an endemic species in different habitats for its various medicinal purposes [10,11]. The preparations of *Kigelia africana* have been shown to possess antimicrobial effects [12] and have cytotoxicity against certain cancer cell lines [13,14]. Other reported ethno-medical uses of this plant include antioxidant activities [15]. Literature on the chemical composition of *Kigelia africana* via bioactivity-guided fractionation has revealed that the extracts contain various cytotoxic agents, such as lapachol (regarded as a potential anti-cancer drug) [16], norviburtinal [13], kigelinone, and  $\gamma$ -sitosterol [17] among others. This study, therefore, investigated the chemotherapeutic effects of various ethanol

extracts of *Kigelia africana* stem bark, fruit and leaf respectively on benzene-induced experimental model of leukaemia in rats using some haematological indices as measuring indicators.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material and Extract Preparation

Fresh stem barks, fruits and leaves of *Kigelia africana* were collected from Kajola farm settlement, Ejigbo Local Government Area, Osun State. The plant with the selected parts were identified at the Botany Department, Obafemi Awolowo University and later authenticated at the Department of Pharmacognosy, Obafemi Awolowo University, Ile-Ife, Osun State. The stem bark was oven dried and coarsely powdered, the leaves were air dried and powdered. The fruits were washed, sliced into smaller pieces, oven dried at 40°C and coarsely powdered. 500 g of the plant parts were separately soaked in 1.5 litres of ethanol for 72 hours using the cold soaking/maceration technique and then filtered using Whatman No.1 filter paper. The filtrates were concentrated using rotary evaporator (Stuart Rotary Evaporator model RE:300) with the *Kigelia africana* bark (KAB) yielding 25.2 g (5.04%), *Kigelia africana* fruit (KAF) yielded 40.8 g (8.1%) and *Kigelia africana* leaf (KAL) yielded 30 g (5.8%). They were then stored at refrigerator temperature (2-6°C) until used.

### 2.2 Animals and Experimental Protocol

Ninety-six Wister strain rats weighing between 150 g and 200 g were purchased from the animal house of Ladoke Akintola University of Technology (LAUTECH), Osogbo Campus, Nigeria and utilized for this research. The rats were randomized into four groups consisting of eight animals per group of two replicates of four for each of the different plant extracts (32 rats per extract of the plant) and subjected to standard 12 hour light/dark cycle. Animals were housed in cages and provided water and feed *ad libitum*. The rats were allowed to acclimatize for seven days before the commencement of the experiment and the animals' room temperature was maintained at 28±2°C. Rats were randomly grouped according to the treatment received and were examined to be free of wounds, swellings and infections before the commencement of the experiment. All experimental protocols were conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals.

### 2.3 Administration of Benzene Solution

The Benzene solution used with Cat No 270709 and >99.9% was purchased from sigma Aldrich GmbH (Steinheim, Germany) and diluted in water for injection at a concentration of 1mL of the benzene to 9 mLs of water for injection. Precisely 0.2 mL was administered intravenously through the tail every two days for four weeks. The young rats administered with benzene were allowed to grow under observation and tested periodically until leukaemia developed in significant number of them which were separated for further experimental treatment protocol.

### 2.4 Acute Toxicity of *Kigelia africana* in Mice

The acute toxicity study of *Kigelia africana* was determined according to the method of Sawadogo and colleagues [18]. Forty eight mice were fasted for 16 hours and randomly divided into eight groups of six animals each. Graded doses (100, 400, 800, 1600, 3200, and 6400 mg/kg) of the ethanolic extract of *Kigelia africana* stem bark, fruit and leaves respectively corresponding to groups II, III,IV,V,VI and VII were separately administered to the mice in each test group by means of an oral cannula. The control group representing group I was administered with distilled water (10 mg/kg) only. All animals were then allowed free access to feed and water and observed for a period of 48 hrs for signs of acute toxicity, morbidity and mortality.

### 2.5 Administration of the Plant Extract

Ninety-six rats were randomized into groups, consisting of eight animals in each group. Group A; leukaemia control rats were administered benzene (0.2 mL) 48-hourly for four weeks. Groups B received commercial feed with water only, C received Benzene and treatment with ethanol extract of *K. africana* for 3 weeks daily, and D received ethanol extract of *K. africana* plant with commercial diet only (different extracts of *K.africana* stem barks, *K. africana* fruits and *K.africana* leaves) respectively. The ethanol extracts of the various *K. africana* plant was administered by gavage once daily with the aid of oral cannula; the dose administered to the appropriate experimental rats was 0.5 mL of 100 mg/mL stem bark, 0.5 mL fruit and 0.2 mL of the leaf extract for 3 weeks respectively.

**Table 1. Experimental protocol**

Group	Treatment	Inference
A (n=8)	Leukaemia induction with benzene only	Leukaemia positive control
B (n=8)	Commercial feed with water only	Leukaemia negative control
C (n=8)	Leukaemia induction with Benzene and treatment with Ethanol extract of * <i>K. africana</i> plant for 3 weeks daily	Chemotherapeutic effect
D (n=8)	Ethanol extract of * <i>K. africana</i> plant with commercial diet only	Adverse reaction / toxicity

\*Ethanol extracts of *K. africana* stem bark, fruit and leaves were investigated separately using the same protocol in 2 replicates each

## 2.6 Sample Preparation

The rats were sacrificed 12 hours after the conclusion of the experiment under light ether anesthesia by cervical dislocation. The blood samples were collected from the inferior vena cava by the use of 5 mL syringe and dispensed into ethylene diamine tetra acetic acid (EDTA) vials, gently mixed and labeled appropriately.

## 2.7 Determination of Haematological Parameters

Haematological parameters and indices were evaluated by flow cytometry (direct current method) using auto-analyzer Outra O SH800-Plus with the aid of suitable cell packs as described by Akinbo et al. [19]. Differential WBC count was also conducted to complement the automation estimation result using Leishman staining technique as previously described by Bain and Lewis [20].

## 2.8 Statistical Analysis

Data were expressed as mean  $\pm$  S.D of two replicates in each group. Analysis of variance (ANOVA) and paired t-test were carried out to test for the level of extract efficacy at  $p < 0.05$  among the groups using the statistical package for social sciences (SPSS) 21.0 versions.

## 3. RESULTS

### 3.1 LD<sub>50</sub>/Acute Toxicity Determination

The oral administration of graded doses (100, 400, 800, 1600, 3200 and 6400 mg/kg) of the ethanol extracts of *K. africana* to mice did not produce any significant changes in behaviour, breathing or gastrointestinal effects during the observation period. Also, no deaths were recorded at any point in this study. The LD<sub>50</sub> of the different parts of *Kigelia africana* is therefore greater than 6400 mg/kg orally in mice.

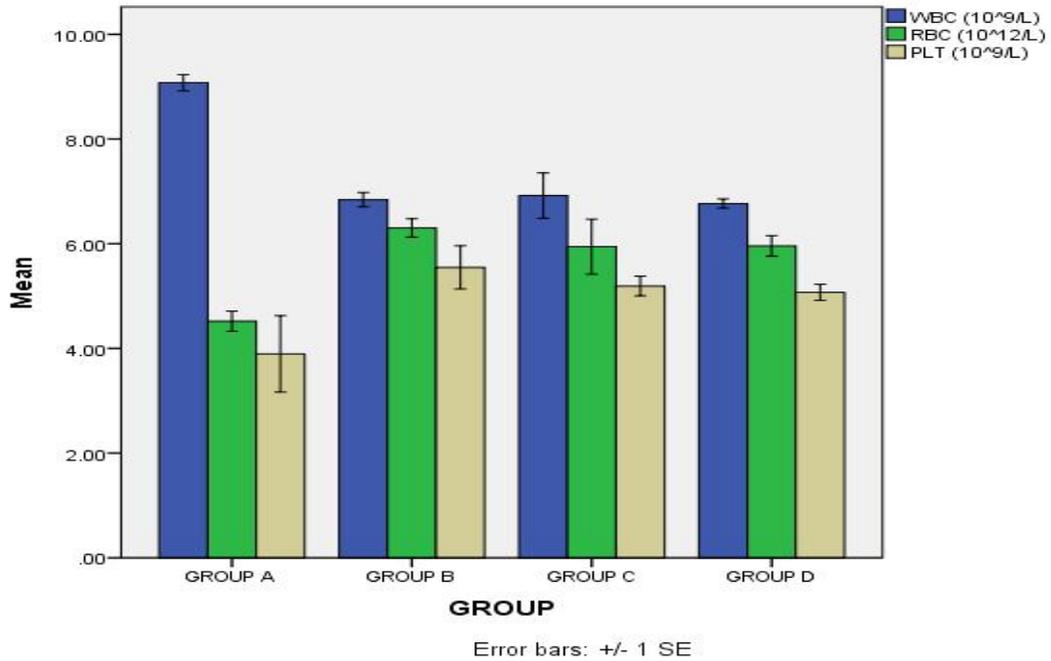
Haematological parameters such as haematocrit (HCT), total white blood Cell (WBC), red blood cell (RBC), haemoglobin (HGB), platelets (PLT), and red cell indices such as mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were assessed in the whole blood of experimental rats (Figs. 1-3). Results of the haematological parameters being the major leukaemic indices in the various experimental groups are shown in Table 2. There was marked reduction in the HCT, RBC, HGB and PLT in group A (Leukaemia Positive Control) while the WBC count was markedly increased when compared to other groups indicative of a successful leukaemia development (Figs. 1 and 2).

The RBC indices were significantly ( $p < 0.05$ ) increased in the treatment groups than the leukaemia positive control group. *K. africana* treatment of the appropriate groups resulted in significant ( $p < 0.05$ ) reduction in the WBC count suggestive of the reversal and alleviation of the leukaemia induction in the treatment group (Table 2).

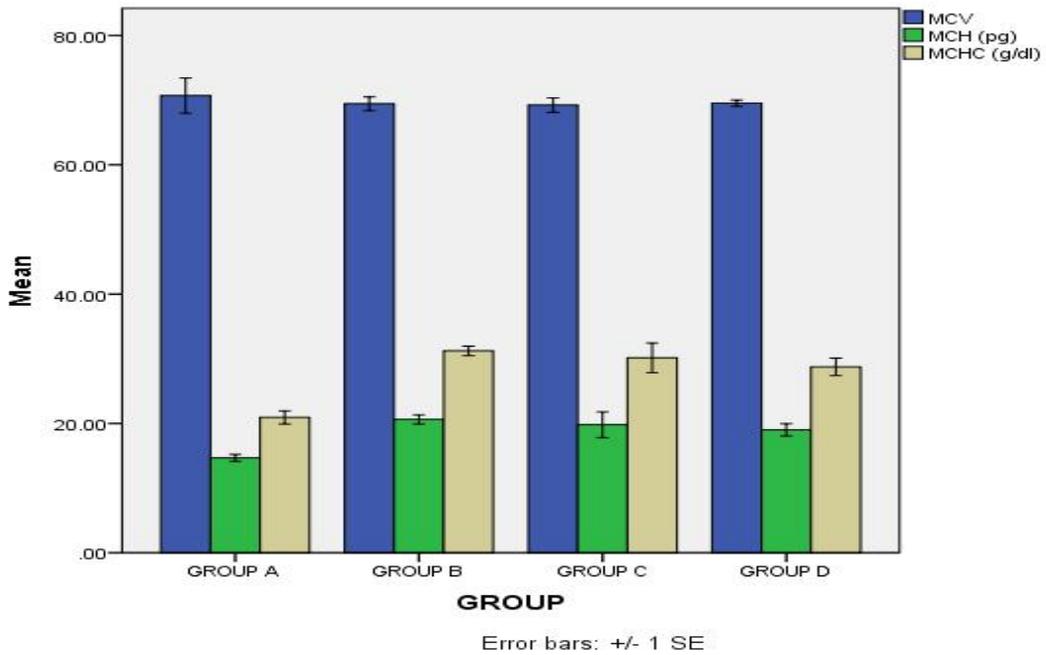
There were significant ( $p < 0.05$ ) differences in all the haematological parameters evaluated in group B (Commercial feed with water only) when compared with A (leukemia induction/positive control) showing significantly elevated leukocyte count and reduced haemoglobin, and packed cells volume in the leukaemia positive group except in platelets and MCV. This confirms that the administered benzene actually induced leukemia in the rats.

Treatment of the healthy rats with all the different ethanol extracts of *K. africana* alongside commercial diet alone (group D) exhibited no significant ( $p > 0.05$ ) variation in the evaluated haematological indices when compared with the healthy rats in group B that received commercial feed and water only (Table 2). This revealed that there was no adverse reaction or toxicity

experienced by the rats as a result of administration of the various ethanol extracts. The estimated parameters in group B served as reference values.



**Fig. 1. Some haematological parameters of the respective groups of experimental rats**  
 Bars represent mean  $\pm$  SEM (n=8). Bars with different statistical markers are significantly different at  $p < 0.05$



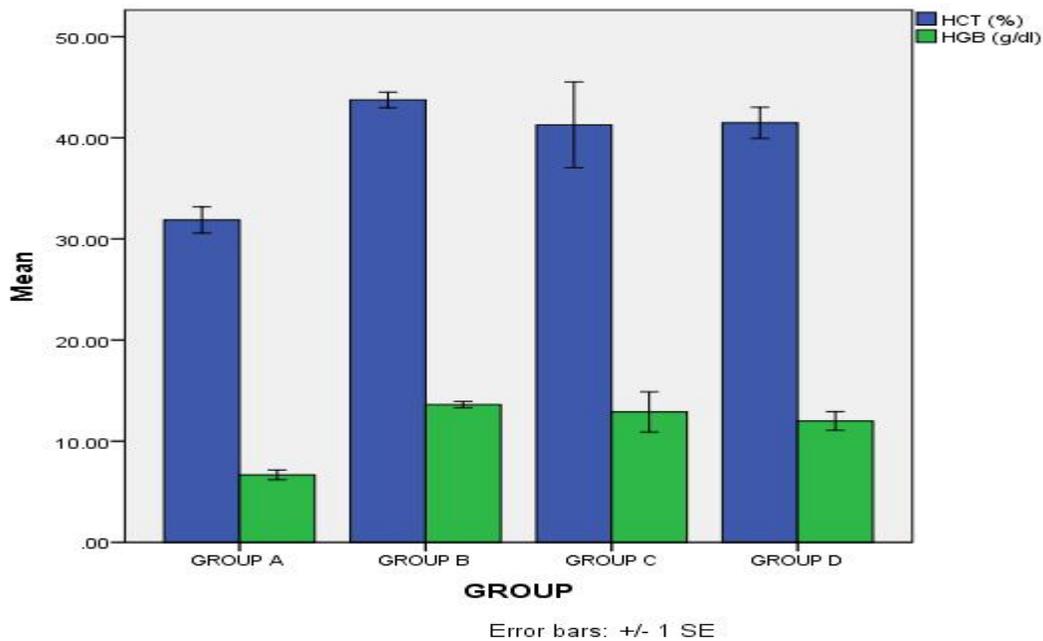
**Fig. 2. Typical haematological indices of the respective groups of experimental rats**  
 Bars represent mean  $\pm$  SEM (n=8). Bars with different statistical markers are significantly different at  $p < 0.05$

**Table 2. Haematological parameters in the various treatment rat groups using the different extracts of *K. africana* plant**

Extract	Group	HCT (%)	WBC ( $10^9/L$ )	RBC ( $10^{12}/L$ )	HGB (g/dL)	PLT ( $10^5/\mu L$ )	MCV (fl)	MCH (pg)	MCHC (g/dL)
KASB	A	31.88±2.59	11.08±0.31	4.52±0.39	6.68±0.96	3.90±1.46	70.70±5.48	14.67±1.09	20.92±2.03
KASB	B	43.74±1.72*	6.84±0.30*	6.30±0.39*	13.62±0.69*	5.19±0.42	69.24±2.50	20.62±1.56*	31.22±1.64*
KASB	C	41.28±9.48	6.92±0.97*	5.94±1.17	12.90±4.45	5.19±0.42	69.24±2.50	19.80±4.45	30.14±5.15*
KASB	D	41.48±3.78*	6.77±0.22*	5.96±0.48*	12.00±2.26*	5.96±0.48	69.53±1.19	19.02±2.34*	28.75±3.29*
KAF	A	31.88±2.59	11.08±0.31	4.52±0.39	6.68±0.96	3.90±1.46	70.70±5.48	14.67±1.09	20.92±2.03
KAF	B	43.74±1.72*	6.84±0.30*	6.30±0.39*	13.62±0.69*	5.19±0.42	69.24±2.50	20.62±1.56*	31.22±1.64*
KAF	C	39.04±7.37	7.40±1.01	5.57±1.01	9.54±4.07	5.13±1.43	69.24±2.50	19.80±4.45	30.14±5.15*
KAF	D	46.88±6.27*	6.77±0.22*	6.68±0.48*	13.43±3.07*	5.46±1.48	69.53±1.19	19.02±2.34*	28.75±3.29*
KAL	A	31.88±2.59	11.08±0.31	4.52±0.39	6.68±0.96	3.90±1.46	70.70±5.48	14.67±1.09	20.92±2.03
KAL	B	43.74±1.72*	6.84±0.30*	6.30±0.39*	13.62±0.69*	5.19±0.42	69.24±2.50	20.62±1.56*	31.22±1.64*
KAL	C	42.02±1.18	6.70±0.55	5.93±0.56	12.18±2.91	4.71±0.29	71.78±2.92	19.34±2.80	30.14±5.15*
KAL	D	40.12±3.27*	6.98±0.18*	5.70±1.81*	13.78±1.29	4.65±0.97	68.36±4.68	18.84±3.79*	28.75±3.29*

Results are expressed as Mean ± S.D. KASB = *Kigelia africana* stem bark, KAL = *Kigelia africana* leaves, KAF = *Kigelia africana* fruit.

\*\* Indicates significant difference at  $p < 0.05$



**Fig. 3. Haematocrit and haemoglobin levels of the respective groups of experimental rats**  
 Bars represent mean  $\pm$  SEM ( $n=8$ ). Bars with different statistical markers are significantly different at  $p < 0.05$

#### 4. DISCUSSION

The modulating effect of the different ethanol extract of *K. africana* plant on some basic haematological parameters was analyzed by assessing circulating levels of total WBC counts, PCV/HCT, platelets count, red blood cell counts, haemoglobin and haematological indices in the peripheral blood of control and treatment groups. Anaemia of chronic disease was observed in rat groups where leukaemia was induced due to exposure to benzene solution which is a potent carcinogen. This anaemia is evident by the significant ( $P < 0.05$ ) reduction in HCT, HGB and red cell counts observed in rats in group A when compared with group B (reference value). Anaemia and thrombocytopaenia are considered some of the classical pointers of leukaemia and other haematological malignancies [21]. These established the successful induction of leukaemia in the appropriate groups especially the positive control group. The reversal of these major anaemia indices in the *K. africana* - treated groups suggests that this extract has the ability to enhance the erythropoietic activities in animal models with leukemia and subsequently ameliorating the condition. This study represents an outcome of the *in-vivo* activity of the ethanol extract of *K. africana* stem barks, fruits and leaves.

Benzene-induced rat leukaemia are a suitable experimental animal model for evaluating the anti-leukaemic effect of natural products and are believed to be predominantly mediated via metabolites such as benzene oxide [22]. The central chain of events in the leukaemia-induction concept is based on the idea of marked leukocytosis precipitated by the reactive metabolites of benzene being able to mutate a critical gene or set of genes related to proliferation and differentiation in human stem cells resulting in chromosomal aberrations (aneuploidy, translocations, inversions, and deletions), aberrant mitotic recombination, gene mutations, and/or epigenetic alterations [23]. This was observed in the groups of leukemia-induced rats as reported also in other previous works [24,25]. The leukaemia-positive control groups (benzene treated only) in this study showed significantly higher total WBC count than the negative control ( $P < 0.05$ ) and treatment groups correlating with the studies listed above. Evidently, the *K. africana*-treated groups showed a significant reduction in the total WBC count evidence of the extract activity. This is consistent with the findings of other previous investigators [13], where they discovered the crude dichloromethane extracts of stem bark and fruit of the plant showed cytotoxic activity *in vitro* against cultured melanoma and other cancer cell

lines using the Sulphorhodamine B assay, which was employed for bioassay-guided fractionation. The *in vitro* cytotoxic activity found in root bark extract of *K. africana* has been attributed to a few of its metabolic compounds most commonly  $\gamma$ -sitosterol which is comparable to standard, lapachol [26]. These results substantiate and support the use of the *K. africana* plant extracts as a potent cytotoxic or anticancer agent.

This study showed progressive improvement in the values of HCT, HGB, PLT, MCV, RBC and MCH of the treatment groups when compared to the leukaemia positive control group. This is indicative of the chemotherapeutic activity of the ethanol extract of the plant against leukaemia and is similar to the results obtained in an earlier study where the effect of *Kigelia africana* and 5-Fluorouracil on the body weight of tumor-bearing mice was assessed which showed that the pure compound of *Kigelia africana* exhibited significant anticancer activity when compared with the standard anti-cancerous drug [27,28]. This finding correlates with other previous studies on plant derived anti-leukaemia treatment which revealed the cytotoxicity of ethanol fractions of *Moringa oleifera* on acute myelogenous leukaemia cell culture [29]. The extracts of the plant have been shown to possess various potential anticancer agents [30, 31]. The anti-leukaemic activity however appears to be highest in the plant stems bark, and least in the leaf.

Further comparison of the *K. africana* treatment groups with the leukaemia negative control group showed that there were no significant ( $P>0.05$ ) differences in the haematological parameters. Similarly, when rats that were administered with the different ethanol extracts of *K. africana* (group D) were compared with groups fed with commercial feed and water only (group B) there was no significant ( $P>0.05$ ) difference in the haematological parameters. This was an indication of the tolerable nature of the bioactive components of the plant extract on the animals [32,33]. This finding indicates that the ethanol extract of *K. africana* has no toxic effect on the red blood cell parameters i.e. red blood cell count, haemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin concentration and mean corpuscular hemoglobin; also that the administration of *K. africana* plant extract to rats does not produce any deleterious effects on red blood cells and haemoglobin. Therefore, the

ethanol extract of *K. africana* has no adverse or toxic effects on healthy haemopoietic cells.

## 5. CONCLUSION

This study, therefore, shows that ethanol extract of *K. africana* stem barks, fruits and leaves possess anti-leukaemic properties as reflected on benzene-induced leukaemia in Wister rats and thereby mitigating the associated anaemia of chronic disease and thrombocytopaenia when administered orally to rats after exposure to benzene. In the future, phytochemical screening and characterization of the extracts of the various plant parts would help determine its bioactive components as our study suggests that the extract might be a promising natural, non-toxic anticancer agent.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

This study was approved by the Experimentation Ethics committee on animal use of the College of Medicine, Ladoke Akintola University of Technology (LAUTECH, Reference no. LAU/OSG/380/VOL.XXXI/72). Also, protocols were conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (1985).

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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