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Anti-leukemogenic Efficacy of *Aloe vera* Gel on Benzene-induced Leukemia in Wistar Rats

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Backgroud: The toxicity of benzene leading to leukemia induction has been well documented in animal model. *Aloe vera* is a succulent perennial evergreen flowering plant used traditionally in the treatment of jaundice and was found to have potent cytotoxic effect against HL60 human acute myeloid leukemia. The present study investigated the *in vivo* chemoprotective effects of *Aloe vera* gel on benzene-induced leukemia in rats.
Methodology: Leukemia was induced in male Wistar rats of 80-90g weight by intravenously administered 0.2ml benzene solution alternate days for four weeks. Following induction, leukemic rats and normal baseline control rats were randomly assigned into four experimental groups of 6 animals each as follows: Group CTRL (control), normal baseline control rats; Group AVG (*Aloe vera* gel), normal baseline rats treated with A*loe vera* gel (150 mg/kg) for 7 days, Group LKR (leukemic rats), untreated leukemic rats serving as leukemia control and Group LKR + AVG, leukemic rats treated with *Aloe vera* gel (150 mg/kg) for 7 days.
Results: Leukemic rats showed altered hematology and morphological deformations such as anisocytosis, poikilocytosis and blast cells occurrence in peripheral blood. Also hypercellularity, severe dysplasia and significantly elevated micronucleated polychromatic erythrocyte were observed in marrow of leukemic rats. Moreover, benzene caused a significant elevation in plasma level of advanced oxidation protein products (AOPPs) with concomitant reduction in total sulfhydryl

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and arylesterase activity. However, treatment with *Aloe vera* gel restored blood hematology to near normal and mitigated the deformities in blood cell morphology induced by benzene. *Aloe vera* supplementation also effected a disappearance of dysplasia and diminution in the frequency of micronucleus in the bone marrow of treated leukemic rats. It also enhanced plasma antioxidant capability by restoring sulfhydryl content and arylesterase activity of the blood and abrogated the increase in plasma content of AOPPs.

Conclusion: Overall, *Aloe vera* gel offered chemoprotective effect on Benzene-induced leukemia in rats.

Keywords: Benzene; leukemia; Aloe vera gel; chemoprotective; antioxidant.

1. INTRODUCTION

Globally, cancer constitutes a major public health problems as it is a leading cause of death and reduction in life expectancy across the world [1]. The rate of occurrences and mortality linked to cancer continue to rise. Estimated value of 19.3 million new cancer cases including non-melanoma and 10.0 million cancer deaths were reported worldwide where leukemia was 10th most commonly diagnosed cancer among male and ranked 8th leading cause of male cancer mortality by global cancer statistics in 2020 [2]. Leukemia is a malignant hematopoietic disease with characteristic feature of uncontrolled proliferation and blockage in differentiation of hematopoietic cells resulting to overproduction of immature blood cells knowns as blasts that enter the blood stream [3]. Leukemia is majorly a cancer of the white blood cells however any of the blood forming cells from the bone marrow can turn into a leukemia cell [4]. Leukemogenesis in human and animal models have been linked to certain etiological factors such as genetic alteration, ionizing radiation, immune deficiency and exposures to some carcinogenic chemicals such as benzene and 7, 12-dimethyl benz[a]anthracene [5,6,7]. Benzene is a feeder chemical in the industry that has been classified as a group 1 carcinogen and specifically known to be leukemogenic to humans [8,9]. Exposure to benzene has been implicated in hematological imbalances that resulted into plastic anemia and leukemia cases especially acute myelogenous leukemia [10,11]. The toxicity of benzene is linked to its bioactivation to reactive intermediates that are capable of increasing the reactive oxygen species [12].

Despite improved scientific research that increased the efficacy of cancer treatment and management [13], multimodality treatment of cancer with certain synthetic chemotherapies such as cyclophosphamide and doxorubicin among others resulted in certain side effects that lead to specific organ toxicity and even secondary malignancy such as therapy-related myelodysplastic syndrome and acute myelogenous leukemia [14,15,16]. Therefore, there is need for more organ friendly herbal medicine with sufficient chemotherapeutic effect. *Aloe vera* is a species of stemless and succulent plant that belongs to the *Liliaceae* family utilized in herbal medicine to treat burns, cough, skin infections, rheumatism, uro-genital and intestinal problems. *Aloe vera* gel together with its main phytoconstituent; emodin, were reported to possess antioxidant, anticancer and anti-proliferative effects on human breast cancer cells [17], gastric cancer cells [18], human hepatoma cells [19] and neuroblastoma [20]. Moreover, *in vitro* cytotoxic and apoptotic activity of *Aloe vera* had been reported against breast carcinoma MCF-7 and HL60 acute myeloid leukemia cells [21]. However, there is no information on *in vivo* anti-leukemia effect of *Aloe vera* and its phytoconstituents. The objective of this study was to determine the effects of *Aloe vera* gel extract in the treatment of leukemia in rats. Therefore, this work was designed to evaluate the chemo-protective effect of *Aloe vera* leaf gel extract on benzene-induced leukemia in male Wistar rats.

2. MATERIALS AND METHODS

2.1 Plant Collection and Preparation of Extract

The whole plant of *Aloe vera* was harvested from *Aloe vera* garden and identified at Herbarium unit of the Department of Botany, University of Ibadan. The leaves of *A. vera* were washed with tap water and cut-open longitudinally at the middle using a clean knife. The gel was collected

by scraping green leaf using knife. The gel was then blended to homogenize it and the homogenate was filtered using a muslin cloth. The filtrate was lyophilized using freeze dryer machine. The resulting solid mass was later reconstituted in distilled water to prepare *Aloe vera* gel (AVG) concentration (150 mg/kg) used for animal study.

2.2 Experimental Animals

Twenty four adult male Wistar strain rats of weight range 80-90g were used for this study. The animals were acclimatized for two weeks in research plastic cages and fed with standard commercial rat feed and clean tap water being supplied *ad libitum*. The present work was conducted with approval of Faculty of Natural Sciences Ethical review of Ajayi Crowther University, Oyo with approval code: Fns/Erc/2019002 and the protocol conformed to the guidelines of the National Research Council for laboratory animal care and use [22].

2.3 Animal Treatments and Groupings

After two weeks of adaptation period, leukemia was induced in twelve Wistar rats (80-90g weight) following the procedure of Akanni *et al*. [23] by intravenous injection of 0.2ml benzene solution (1:5:5 of Benzene/2-propanol/distilled water v/v) every 2 days for 4 consecutive weeks. Following induction, 24 rats comprising 12 leukemic rats and 12 normal baseline control were randomly assigned into four experimental groups of 6 animals each as follows: Group CTRL, normal baseline rats; Group AVG, normal baseline rats treated with A*loe vera* gel (150 mg/kg) for 7 days, Group LKR, rats with benzene-induced leukemia that received 0.2ml intravenous injection of benzene:2-propanol:water mixture (1:5:5 v/v) for four consecutive weeks and Group LKR + AVG, leukemic rats that were treated with *Aloe vera* gel (150 mg/kg) for 7 days.

2.4 Collection of Blood and Bone marrow

After 24 hours of final treatment blood samples were collected from each animal through retro orbitals plexus into lithium heparinized tubes for biochemical assays and ethylene diaminetetraacetic acid (EDTA) bottle for the hematocrit, total white blood cell (WBC) counts, red blood cell (RBC) counts, hemoglobin (Hb), platelets counts estimation using the automated blood analyzer and thereafter sacrificed. The femur bones were excised to obtain bone marrow for micronucleus assay and hematoxylin and eosin staining for histopathological examination.

2.5 Assay for Oxidative Stress Makers in the Plasma

Plasma AOPP was determined by the method described by Witko et al. [24] as modified by Zhang et al. [25] Briefly, plasma (100µl) was added to 400µl of phosphate buffer saline (PBS) solution and 25µl 1.16M potassium iodide (KI) was then added followed 2min later by 50µl of acetic acid. The absorbance of the reaction mixture was immediately read at 340nm against a blank containing 500µl of PBS, 25µl of KI, and 50µl of acetic acid. Plasma total thiol were measured spectrophotometrically using DTNB (2, 2'-dinitro-5, 5'-dithiodibenzoic acid) [26]. Arylesterase activity was determined using phenylacetate as the substrate following the procedure described by Erdem et al. [27].

2.6 Micronucleus Assay

Clastogenicity in leukemic rats were evaluated in the bone marrow of the rats employing the micronucleus assay techniques as described by Heddle and Salmone, [28] with modification by Heddle, et al. [29]. Briefly, Bone marrow from femurs of rats were used for preparation of slides using standard procedure Matter and Schmid [30]. A pair of scissors was used to make an opening in the iliac region of the femur. A small pin was then introduced into the marrow canal at the epiphyseal end to exude marrow through the hole at the iliac end into a slide. A drop of fetal calf serum was added to the smear and was mixed to become homogeneous by using a clean edge of another slide. The homogeneous mixture was then spread on the slide as a smear and allowed to dry. The dried slides were fixed in absolute methanol, air-dried and then pretreated with May-Grunwald solution which was subsequently air-dried. The already air-dried slides were stained in 5% Giemsa solution and sequentially rinsed for 30s in both phosphate buffer and distilled water and then air-dried. Finally the slides were fixed in xylene for 20 minutes and air-dried and thereafter mounted and scored for micronucleated polychromatic erythrocytes (PCEs). For each experimental

animal, two slides were prepared and 500 PCEs were scored blindly from each slide viewing through different fields (at least 5 fields) of each slide and summed together to determine number of MnPCEs in total of 1000PCEs scored for each rat.

2.7 Statistical Analysis

Data are presented as the mean ± standard deviation (SD) of six replicates. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Duncan's multiple comparison between control and treated rats in all groups using SigmaPlot® statistical package (Systat Software Inc., San Jose, CA, USA). *P*-values less than *0.05* ($P < 0.05$) were considered statistically significant.

3. RESULTS

3.1 Influence of *Aloe vera* Gel on Hematological Parameters and Blood Morphology of Benzene-induced Leukemic Rats

The Influence of *Aloe vera* gel on hematological parameters and blood morphology of benzene-induced leukemic rats was shown in Table 1. Leukemic rats showed a decrease in packed cell volume (PCV), erythrocyte counts, hemoglobin contents and a significant increase in WBC counts when compared to the baseline control. Moreover, blood morphology of the leukemic rats shows the presence of anisocytosis, poikilocytosis and appearance of blast cells in the peripheral blood film that were not noticed in normal baseline control. Upon treatment with *Aloe vera* gel, the disproportion in hematological parameters were normalized with reduction in the percentage blast when compared to untreated leukemic control.

3.2 Influence of *Aloe vera* Treatment on Bone Marrow Architecture of Benzene-Induced Leukemic Rats

The architecture of bone marrow of leukemic rats treated with extract of *Aloe vera* gel was shown in Fig. 1. The normal baseline control (CTRL) and baseline treated with *Aloe vera* gel extract (AVG) show normocellularity in the architecture of their bone marrows. Hypercellularity and severe dysplasia were observed in leukemic rats (LKR). However, there were hypercellurity but with mild dysplasia in *leukemic* rats that were treated with *Aloe vera* gel (LKR + AVG).

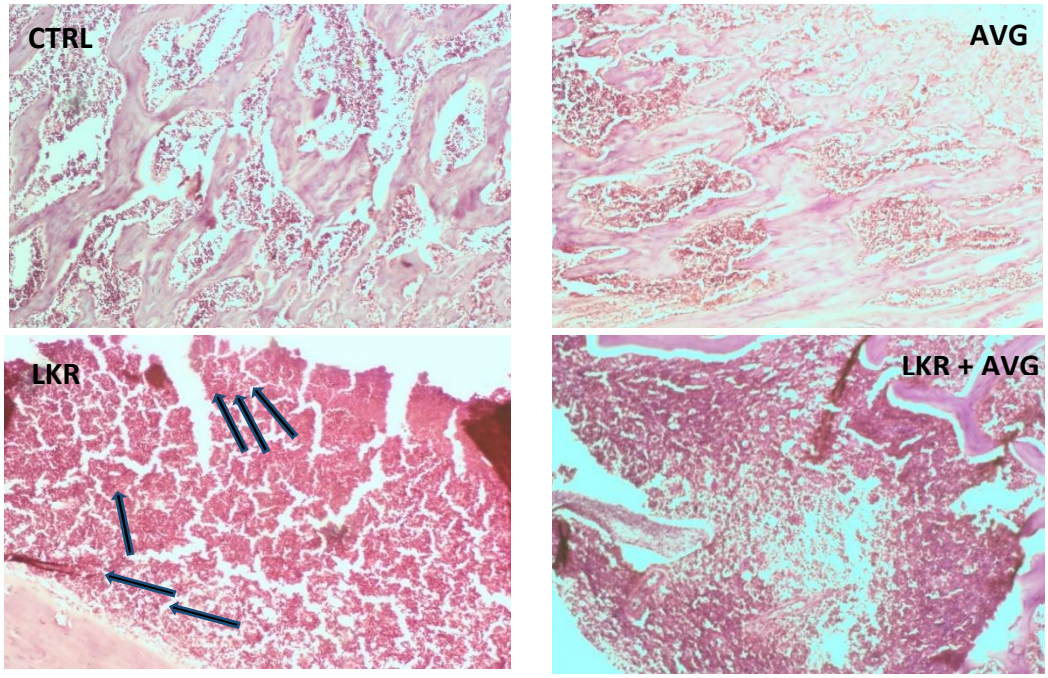


Fig. 1. Photomicrograph of bone marrow of leukemic rats treated with *Aloe vera* gel extract with arrows showing dysplasia

Table 1. Influence of *Aloe vera* gel on hematological parameters and blood morphology of benzene-induced leukemic rats

GROUPS	PCV % (Mean±SD)	HGB g/dl (Mean±SD)	RBC x 10^{12}/L (Mean±SD)	PLT × 10^{9}/L (Mean±SD)	WBC × 10^{9}/L (Mean±SD)	% Blast cells	ANISD	POIK
CTRL	45.33±2.52	15.00±1.00	7.60±0.32	649.67±±57.55	9.17±1.96	-	-	-
AVG	47.33± 2.52	15.30± 0.60	7.91± 0.37	658.67±103.00	9.20±1.77	-	-	-
LKR	41.75±2.22	13.45±0.70	6.98±0.65	807.25±131.00	12.60±2.14	5	++	++
LKR+ AVG	49.00±5.57	15.67±1.86	8.47±0.59	698.67±52.08	9.50±2.66	2	+	+

Abbreviations: PCV, packed cell volume; HGB, hemoglobin; RBC, red blood cell; PLT, platelet; WBC, White blood cell; ANISD, Anisocytosis; POIK, Poikilocytosis

3.3 Effect of *Aloe vera* Gel on Occurrence of Micronucleated Polychromatic Erythrocytes in the Bone Marrow of Benzene-Induced Leukemic Rats

Effect of *Aloe vera* gel on bone marrow concentration of micronucleus assay on Benzene-induced leukemia in Wistar rats was presented in Fig. 2. Leukemic rats showed a significant elevation in the concentration of micronucleus assay present in the bone marrow by 408.23% when compared to the control group. Treatment with *Aloe vera* gel significantly attenuated the effect of benzene-induced genotoxicity by reducing the bone marrow micronucleus occurrence by 53.70% in the bone marrow when compared with the untreated leukemic group (LKR).

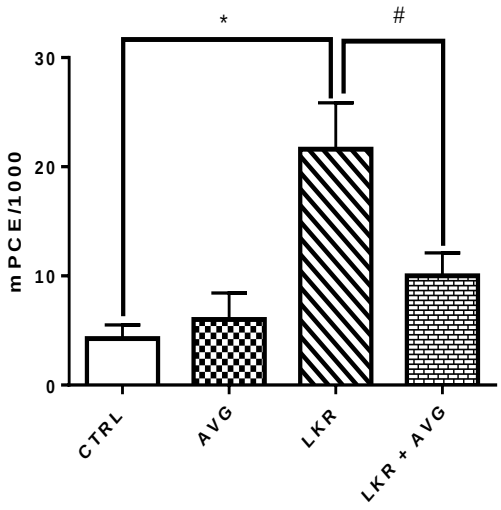


Fig. 2. Effect of *Aloe vera* gel on bone marrow occurrence of micronucleus in Benzene-induced leukemia in wistar rats

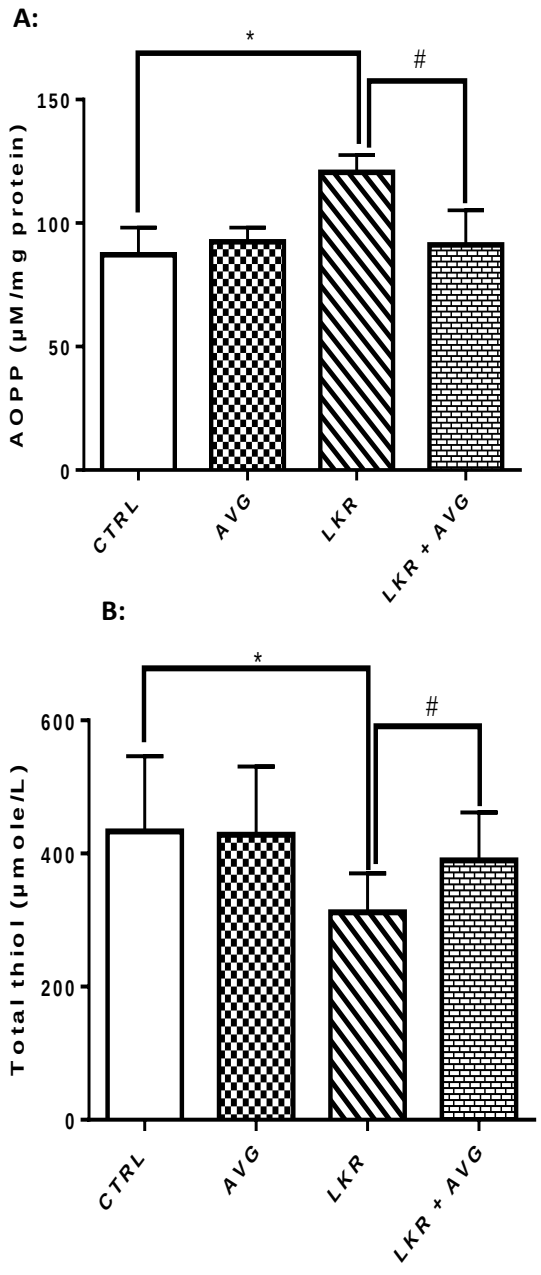
Data are expressed as mean ±S.D for six rats in each group

**significantly different from the control p<0.05*

 # significantly different from LKR group

3.4 Effect of *Aloe vera* Gel on Plasma Oxidative Resistance in Leukemic Rats

The induction of leukemia in rats was accompanied by a significant elevation in the concentration of advanced oxidation protein products present in the blood plasma by 38.18% when compared to the control group (Fig. 3A). However, administration of *Aloe vera* gel significantly attenuated the effect of benzene toxicity by reducing the concentration of plasma AOPP by 24.33% in the plasma when compared with the leukemic control. Also a significant decrease in the level of total thiol content and activity of arylesterase enzyme present in blood plasma by 28.02% and 53.99% respectively were observed when compared to the control group in Fig. 3B and 3C. Treatment with *Aloe vera* gel significantly established an increase in the level of total thiol by 23.02% and arylesterase activity by 35.35% in the plasma when compared with the untreated leukemic rats.



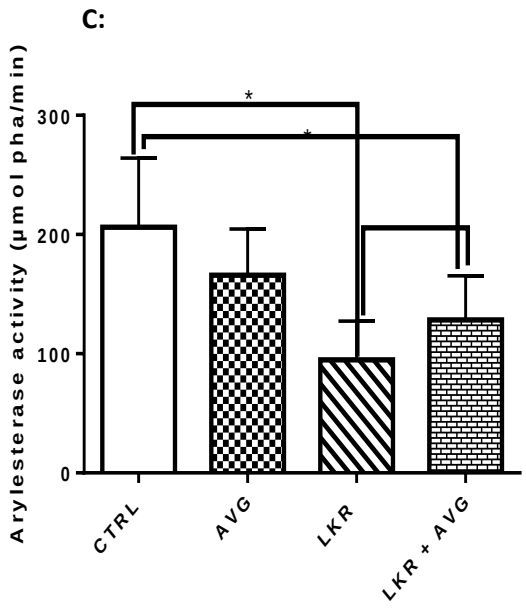


Fig. 3. Effect of *Aloe vera* gel on plasma antioxidant markers: Advanced Oxidation Protein Products-AOPPs (A), Total thiol (B) and plasma activity of Arylesterase (C) on Benzene-induced leukemic rats

Data are expressed as mean ±S.D for six rats in each group

**significantly different from the control p<0.05*

[#] significantly different from LKR group

4. DISCUSSION

Benzene is a known pollutant in the environment that causes harm to the hematopoietic system resulting into aplastic anemia, pancytopenia, increased number of non-resting hematopoietic stem cells and leukemia [31,32,33]. Therefore, hematological disturbance and leukemogenic effect of benzene have been well investigated in animal model where it effected a decreased hemoglobin contents, packed cell volumes and platelets counts [6,34].

The result from this study showed a reduction in RBC count, PCV and Hb level when compared to the values of baseline rats. There are also presence of poikilocytosi and anisocytosis in the leukemic rats relative to the control animals. PCV reflects the circulating red blood cells and indicates the extent of polycythemia and anemia. Animals were considered to be anemic when there is a decreases in PCV, RBC count, Hb level, and observable deformability in erythrocytes [35]. Moreover, leukocytosis depicted by significant increase in white blood cell counts was observed in leukemic rats when compared to the control rats. This corroborated the earlier finding that associated leukocytosis to leukemia [23]. The result also showed an appearance of blasts in the peripheral blood film of the leukemic rats, an unusual occurrence of undifferentiated blood forming cells in the blood. This supported the previous findings of other researchers that reported the appearance of blasts in leukemic rats [7]. However, supplementation with *Aloe vera* gel resulted into restoration to normal of Hb level, total WBC and RBC and concomitant reduction in the percentage blasts in leukemic rat model when compared to leukemia control. Moreover, the cross sectional examination of bone marrow of leukemic rats showed the hypercellularity and severe dysplasia Fig. 1 (LKR). Morphological observation of dysplasia in bone marrow is a common finding that has been associated with newly diagnosed acute myelogenous leukemia [36,37]. However, the administration of *Aloe vera* gel to leukemic rats reduced the dysplasia occurrences in the bone marrow tissue.

The determination of micronucleus frequency in the peripheral blood lymphocytes is an important utility for estimation of chromosomal damage in human populations [38]. Several findings have shown the relevancy of peripheral lymphocyte micronucleus expression in the determination of degree of disease where patients with more micronucleus expression tend to have more malignancy [39,40]. There are few studies about micronucleus testing and prognosis of Leukemia or myelodysplasia. This study investigated the capability of *Aloe vera* to protect benzene-induced genotoxicity using induction of micronucleus in bone marrow as biomarker. Leukemia induction in rats by benzene solution was accompanied with significantly increased formation of micronucleated polychromatic erythrocyte in the bone marrow of the rats when compared to the baseline control rats. This results was similar to findings of other researchers on genotoxic effects of several chemical and biological carcinogens in rat models [41,42]. Administration of *Aloe vera* gel significantly mediated a decrease in micronucleus frequency in leukemic rats supplemented with *Aloe vera* gel when compared with the leukemia control group. This therefore demonstrated genoprotective effect of *Aloe vera* gel in benzene-induced leukemia. This result supports the previous reports on genoprotective

effects of *Aloe vera* against genotoxicities caused by benzo [a] pyrene and ethylmethanesulfonate [43,44].

A link has been established between onco-hematological diseases such as leukemia and oxidative stress [45,46,47]. Advanced oxidation protein products (AOPPs) which are oxidized products of dityrosine-containing protein are biomarker of oxidative stress resulted from chlorinated oxidants and plasma proteins [24,48]. AOPPs are known markers of oxidative protein damage that shows the magnitude of inflammation and oxidative stress [49]. Significantly elevated level of AOPPs have been estimated in some pathological conditions such as chronic kidney disease, diabetes, uremia and rheumatoid arthritis [50,51,52].

The oxidative stress also has effect on reductant function of protein sulfhydryl groups that transport reducing equivalents as they proffer antioxidant effect through thiol groups present on them [53,54]. Therefore, antioxidant status is indicated by plasma levels of protein thiol in the body where the reduced protein thiol content correlated positively with advanced oxidation protein products (AOPPs) [55]. The present work showed an increase in plasma advanced oxidation protein products with concomitant decline in plasma total thiol content in leukemic rats relative to normal control. The increased AOPPs is a product of plasma albumin oxidation which is a consequence of increased production of reactive oxygen species (ROS) that resulted from a distorted balance between antioxidants and pro-oxidants like hypochlorous acid and reactive oxygen species [56,57]. The result as shown in Fig. 3B revealed that there was a significant reduction in sulfhydryl protein with concomitantly significant elevated AOPP concentration (Fig. 3A) in leukemic rats when compared to baseline control. Reduced levels of protein thiols was earlier reported to correlate negatively with the levels of AOPPs [58] which is in consonant with result of this present work. The generation of AOPP in this study suggests the induction of oxidative stress due to benzene biotransformation that consequently led to oxidant-mediated protein damage [59]. However, administration of *Aloe vera* gel in this study significantly reduced the elevated AOPP level and effectively restored the total thiol status to near level of normal control when compared to leukemia control group. This suggests the preserving capability of plasma sulfhydryl protein contents by *Aloe vera* gel.

Long term of intense oxidative stress can result into carcinogenesis as oxidative stress may take part in cell proliferation process, malignant transformation, cause damages to DNA and other biological molecules leading to occurrence of tumors [60]. Thus, cancer is a disease of cells that is caused by combined environmental and genetic factors [61]. The arylesterase activity of paraozonase-1 is a gene product of PON1 gene located on chromosome 7q 21.3 with antioxidant properties [62,63]. Decreased serum activity of PON1 has been reported in some cancer patients [64]. The polymorphism of PON1 gene has been used as a prediction for cancer susceptibility due to its ability to detoxify the products of oxidative stress [65] and considered a risk factor for the occurrence of certain cancer types like breast, prostate and hematologic cancers [66]. In the present study, there was a significant reduction in the plasma activity of arylesterase in the leukemic rats which may be due to overwhelming pro-oxidant effect of benzene intoxication leading to oxidative stress condition. However, there was a relative increase in the arylesterase activity upon treatment of leukemic rats with *Aloe vera* gel when compared with leukemia control group in Fig. 3C. Plasma arylesterase possesses lipophilic property and had been shown to be involved in hydroperoxide hydrolysis and reduction in the accumulation of peroxidation products [67,68]. This result suggested that *Aloe vera* may improve plasma activity of arylesterase antioxidant enzyme.

5. CONCLUSION

Overall, *Aloe vera* gel offered chemoprotection against benzene-induced leukemia in rats via its protective roles on hematology, myeloid tissue and its plasma antioxidant buffering capability. This may be due to its antioxidant capability.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The present work was conducted with approval of Faculty of Natural Sciences Ethical review of Ajayi Crowther University, Oyo with approval code: Fns/Erc/2019002 and the protocol

conformed to the guidelines of the National Research Council for laboratory animal care and use.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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