Chemotherapeutic potentials of aqueous *Securinega virosa* leaf extract in benzene-induced leukaemic mice

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ABSTRACT

Background: *Securinega virosa* (common bushweed; Chinese waterberry) is a widely distributed plant throughout tropical Africa and China, but can also be found in India, Malaya, and Australia. The leaves are used traditionally to treat fever, body pain, stomach ache, rheumatism, epilepsy, infectious and chronic diseases including diabetes and cancer due to the presence of phytochemicals of therapeutic importance.

Objective: This study investigated the anti-leukaemic and chemopreventive properties of aqueous *Securinega virosa* leaf extracts in benzene-induced leukaemia.

Methods: Forty male Swiss mice (weight range 15-32g) categorised into control (8) and test (32) groups were used. Leukaemia was induced in the test group using 400 mg/kg body weight benzene. Blood film was prepared and examined microscopically for the presence of blast cell to confirm leukaemia. For therapeutic studies, *Securinega virosa* extract was administered for a period of three weeks after blood was obtained for haematological studies and film preparation. Bone marrow extraction and liver tissue excision were done for microscopic and histopathological studies. Liver tissue malondialdehyde (MDA) and catalase levels were also determined.

Results: showed the presence of blast cells in the blood and BM; and revealed a significant increase ($p < 0.05$) in percentage blast cells, percentage micronucleated polychromatic erythrocytes, leukocyte counts, erythrocyte count and liver MDA; and a significant decrease ($p < 0.05$) in haemoglobin concentration, haematocrit, platelet count, and liver catalase activities amongst leukaemic-untreated groups when compared to *Securinega virosa* extract-treated groups.

Conclusion: The anti-leukaemic potentials and chemopreventive activity of aqueous *Securinega virosa* extract is hereby inferenced as indicated by improvements in haematological parameters, markers of oxidative stress, and the general liver cyto-architecture described by well-organised portal triad and well distributed hepatocytes among the extract-treated mice.

Keywords: Benzene, leukaemia, oxidative stress, antioxidants, haematological parameters

Potentiel chimiothérapeutique de l'extrait aqueux de feuilles de *Securinega virosa* chez des souris leucémiques induites par le benzène

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RÉSUMÉ

Contexte: *Securinega virosa* (épine-vinette commune; épine-vinette à feuilles de Chine) est une plante largement répandue en Afrique tropicale et en Chine, mais on la trouve également en Inde, en Malaisie et en Australie. Les feuilles sont traditionnellement utilisées pour traiter la fièvre, les douleurs corporelles, les maux d'estomac, les rhumatismes, l'épilepsie, les maladies infectieuses et chroniques, notamment le diabète et le cancer, en raison de la présence de substances phytochimiques d'importance thérapeutique.

Objectif: Cette étude a examiné les propriétés anti-leucémiques et chimio-préventives de la solution aqueuse des extraits de feuilles de *Securinega virosa* dans la leucémie induite par le benzène.

Méthodes: Quarante souris mâles suisses (poids compris entre 15 et 32 g) regroupées en groupes témoin (8) et test (32) ont été utilisées. La leucémie a été induite dans le groupe test avec 400 mg/kg de poids corporel de benzène. Un frottis sanguin a été préparé et examiné au microscope pour détecter la présence de cellules blastiques afin de confirmer la leucémie. Pour les études thérapeutiques, L'extrait de *Securinega virosa* a été administré pendant une période de trois semaines après le prélèvement de sang pour les études hématologiques et la préparation du film. L'extraction de la moelle osseuse et l'excision du tissu hépatique ont été effectuées pour des études microscopiques et histopathologiques. Les taux de malondialdéhyde (MDA) et de catalase du tissu hépatique ont également été déterminés.

Résultats: ont montré la présence de cellules blastiques dans le sang et la moelle osseuse; et ont révélé une augmentation significative ($p < 0,05$) du pourcentage de cellules blastiques, du pourcentage d'érythrocytes polychromatiques micro-nucléés, du nombre de leucocytes, du nombre d'érythrocytes et du taux de MDA dans le foie; et une diminution significative ($p < 0,05$) de la concentration d'hémoglobine, de l'hématocrite, du nombre de plaquettes et des activités de catalase hépatique parmi les groupes leucémiques non traités par rapport aux groupes traités avec l'extrait de *Securinega virosa.*

Conclusion: Le potentiel anti-leucémique et l'activité chimio-préventive de l'extrait aqueux du *Securinega virosa* sont donc confirmés par l'amélioration des paramètres hématologiques, des marqueurs du stress oxydatif et de la cyto-architecture hépatique générale décrite par une triade portale bien organisée et des hépatocytes bien répartis parmi les souris traitées à l'extrait.

Mots clés: Benzène, leucémie, stress oxydatif, antioxydants, paramètres hématologiques,

INTRODUCTION

Leukaemia is a form of white blood cell malignancy characterised by transformation of hematopoietic progenitors and diffuse bone marrow infiltration, resulting in the production of abnormal immature blast cells.[1] It is associated with the presence of precursor cells of various lineages, mature cells, or both precursor and mature cells in the peripheral blood. Though exact cause is unknown, factors such as age, gender, environmental, genetic predisposition, smoking, ionising radiation, prior chemotherapy, genetic disorders like Down syndrome, and exposure to chemicals like benzene are considered as risk factors.[2,3] Based on data obtained from cases of leukaemia and deaths from the disease, the rate of new cases and mortality was estimated as 14.0 per 100,000 and 6.0 per 100,000 respectively, while approximately 1.6 % of the population was predicted to be diagnosed of leukaemia at some point during their lifetime.

Tracking of new cases, deaths, and survival over a period of time will facilitate understanding of the progression made, identify and address challenges including improving disease screening and finding better treatments. Statistical models had revealed a decline in age-adjusted new leukaemia cases and mortality rates on the average of 0.6 % and 2.0 % per year respectively and projected that there will be 59,610 (3 %) new cases with 23,710 (3.9 %) death in 2023.[4,5] However, the overall survival of leukaemic patients has remarkably improved due to advance therapeutic strategies and discovery of new drugs.[6]

Management involves supportive and palliative cares in addition to chemotherapy, radiotherapy, targeted therapy involving administration of tyrosine kinase inhibitors, and bone marrow transplant, which are used singly or combined, depending on the type of leukaemia and age of the affected individuals. Though advancements in treatment modalities have increased the 5-year survival rate to 66.7%,[5] associated complications from either the ailment or toxic effects of these drugs, and cost-driven limited access to available treatment modalities, especially among people living in low-income countries, had resulted into increased search for traditional means of treatment. Alternatively, plant-based secondary metabolites have been used to treat various diseases either in their crude, pure extracted (using polar and non-polar solvents) or standardised forms due to their efficacy, readily availability, chemical diversity, and provisions for new drug discoveries.[7] The use of herbal remedies for primary health care needs in Africa and Asia is dated back to stone ages and depicts a long-term history of human-environment interactions. Discoveries of such plants are occasionally made through accidental observational findings of animal-plant interactions, especially by hunters, which were cautiously extrapolated and deduced for the treatment of human ailments. *Securinega virosa* is one of such plant widely distributed throughout tropical Africa, India, Malaya, Australia, and China where it is commonly ingested as local food called Chinese cuisine in all the thirty-four regions. In Nigeria, it is found in virtually all parts of the country and called various names like 'Awewe or Iranje', 'Itachen-gado or Tsuwawun karee' and 'Njisi nta' respectively amongst the Yoruba, Hausa, and Igbo tribes of Nigeria.

Securinega virosa has been discovered in African and Chinese traditional medicine to have activities against stomach ache, rheumatism, hyperglycemia, infection (fungal, bacterial, plasmodial), diarrhea, and epilepsy, thus described as "versatile" plant.[8-11] Different extracts (aqueous, ethanolic, hexane and ethyl acetate) of Securinega virosa leaves, stem back, and root have been demonstrated to have high antioxidant activities, exhibit anti-inflammatory and analgesic activities and thus used in traditional African and Chinese medicine as sedative in children and adults with mental illnesses and also as antipyretic, anti-ulcer, and anticancer agent.[12-18] This study therefore investigated the anti-leukaemic activities of aqueous *Securinega virosa* and its chemoprotective potentials in mice.

METHODS

Collection of plant and preparation of aqueous leaf extract

Securinega virosa leaves were collected from a pre-desertic region around Ladokun village Ogbomoso, Oyo-State and authenticated in the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso (voucher no LHO 509). The leaves were washed under running water to remove dirt, air-dried in the laboratory at room temperature (22-28^{o}C) for 21 days (as evidenced by a constant weight) and powdered mechanically using an electric blender (Braun Multiquick Immersion Hand Blender, B White Mixer MR 5550 CA, Germany). Exactly 100 g of the powdered leaf was measured into a clean transparent reagent bottle and soaked with 300 mL of distilled water. The mixture was regularly shaken for a period of one week to allow for dissolution of water-soluble phytochemicals therein present after which the mixture was decanted and filtered using Buckner funnel and Watman No. 1 filter

paper. The filtrate was concentrated using rotary evaporator at 50-55^{o}C and freeze-dried. The stock solution was prepared by dissolving 1 g of extract in 10 mL distilled water to give a concentration of 100 mg/mL.

Experimental animals

Forty male Swiss mice (weight range 15-32 g) were obtained from animal house unit of the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology Ogbomoso and acclimatised under ambient temperature range (22-28^{o}C) for one week during which they have access to standard mice feeds obtained from Bova Jay Nig. Ltd, Ogbomoso and water ad libitum. The care of the animals was done in accordance with the U.S. Public Health Service Guidelines.[19] Cages were made of non-toxic plastic materials spacious enough to allow for regular cleaning, animal handlings and prevent mice escape. Animal house is well ventilated, relatively silent from noise, and under the natural cycle of daylight and night darkness.

Induction and confirmation of Leukemia

Leukaemia was induced by intraperitoneal administration of benzene diluted in propanol and water (2:1:1) at a dose of 400 mg/kg body weight every 48 h for four weeks.[20] Leukaemia was confirmed microscopically with the appearance of blast in the peripheral blood using tail blood smear from the mice.

Grouping and treatment

The mice were grouped into five, each consisting of 8 mice as follows:

Group A: 10 mL/kg Distilled water (control group).

Group B: 400 mg/kg body weight benzene + Distilled water (untreated control).

Group C: 400 mg/kg body weight benzene + 100 mg/kg body weight aqueous *Securinega virosa* leaf extract.

Group D: 400 mg/kg body weight benzene + 200 mg/kg body weight aqueous *Securinega virosa* leaf extract.

Group E: 400 mg/kg body weight benzene + 25 mg/kg body weight 5-florouracil (standard anticancer drug).

Mice in the extract-treated groups (Group C and D), were administered with 100 mg/kg and 200 mg/kg body weight of aqueous *Securinega virosa* leaf extract which was instituted after leukaemic features had been established and done for a period of three weeks after which they were sacrificed.

Sample collection and preparation

Forty-eight hours after the last treatment, the mice were sacrificed by cervical dislocation and blood collected by cardiac puncture into ethylene diamine tetra acetic acid (EDTA) bottles for haematology studies.

Bone marrow extraction and blood film preparation

The bone marrow (BM) was extracted[21] from the femur and smeared directly on slides with a drop of bovine calf serum, fixed with 30 % methanol and stained with May-Grunewald's and 5 % Giemsa. The slides were air-dried and blindly analysed microscopically. The percentage of blast cells present in the BM was calculated by dividing the number of blast cells present in the BM by the total number of leukocytes present in the BM x 100.

Also, the collected blood was smeared on a glass slide, air-dried at room temperature, stained with Leishman stain and double diluted with distilled water for 10 minutes. The slide was then rinsed in distill water, air-dried and examined microscopically for the presence of blast cell. The percentage of blast cells present in peripheral blood (PB) was calculated by dividing the number of blast cells present in the PB by the total number of leukocytes present in the PB x 100. The percentage micro-nucleated polychromatic erythrocytes (%MNPCE) was also calculated.

Preparation of liver homogenate and slide

The liver was harvested, washed several times in washing buffer to remove haemoglobin which may inhibit activity of the enzyme, and then weighed. Five microns (5 µ) liver tissue section was excised using a rotary microtome (YSPD-Q508, Infitek, China) and fixed in 10% buffered formalin to prevent autolysis and putrefaction, embedded in paraffin wax, smeared on glass slides, stained with haematoxylin and eosin (H&E), and observed for pathological changes under a binocular microscope (MSC-BH102T; MSC-BS102T, Scitek, US).[22,23] The remaining liver tissue was washed in ice-cold saline, homogenized, transferred into equal amounts of PBS buffer (comprising of 150 mM KCl and 0.1 M phosphate buffers; pH 7.4) and centrifuged at 3000 rpm for 15 min to obtain the supernatant which was stored at 4^{o}C for subsequent determination of malondialdehyde (MDA) concentration and antioxidant enzyme activities. Tissue MDA concentration and catalase enzyme activities were measured using standard methods.[24, 25]

Haematological and biochemical studies

Blood sample in EDTA bottle was used to determine haematological parameters (leukocyte counts, haemoglobin concentration, haematocrit, erythrocyte count and platelet count) using automated haematology analyser.

Histopathological studies

This was done to confirm benzene-induced leukaemic changes and tissue lesions. Bone marrow contains haematopoietic stem cells that produces cellular elements of the blood notably platelets, red blood cells and white blood cells. Realising that the ratio of myeloid series and erythroid cells is relevant to bone marrow function, diseases of the bone marrow (leukaemia) and that of peripheral blood (anaemia), marrow biopsy and aspiration were done to examine the source of blood cells and obtain more information on haematopoiesis.

Ethical approval

Ethical clearance was obtained from the Health Research and Ethics Committee of Ladoke Akintola University of Technology with approval number: LAUTECH/EC/2017/10/012. The experiments complied with the World Medical Association guidelines on animal use in biomedical research and that of the U.S. Public Health Service Guidelines. The animals' rights and welfare were protected.

Statistical analysis

This was done using SPSS package for windows version 25.0. Descriptive statistics was used to describe and represent variables. Differences in mean between the groups were determined using one-way ANOVA. Differences in mean among the groups were considered significant at $p < 0.05$.

RESULTS

Percentage micronucleated polychromatic erythrocytes

Result from this study showed the presence of high proportion of blast cells in the peripheral blood and bone marrow of benzene-treated mice (33% and 22% respectively), unlike a low proportion seen in the controls (3 % and 0 %), 100 mg/kg Securinega virosa dosage (15% and 9.5 %), and 200 mg/kg Securinega virosa dosage (12% and 7 %) groups respectively (Table 1). A significant increase ($p < 0.05$) in %MNPCE value was observed amongst leukaemic untreated mice when compared to aqueous *Securinega virosa* extract-treated (100 mg/kg and 200 mg/kg doses) and 5-fluorouracil-treated groups (Figure 1). Also, a significant decrease ($p < 0.05$) in %MNPCE value was observed in the 200 mg/kg Securinega virosa extract dosage (0.8 ± 0.20) as compared to that of the 100 *Securinega virosa* extract dosage (0.5 ± 0.05).

Chemotherapeutic effect of aqueous *Securinega virosa* leaf extract

Result from this study revealed a significant decrease ($p < 0.05$) in haemoglobin concentration, haematocrit, RBC count, and platelet count in leukaemic untreated mice when compared to aqueous *Securinega virosa* extract-treated (100 mg/kg and 200 mg/kg doses) and 5-florouracil-treated leukaemic mice (Table 1). However, a significant increase ($p < 0.05$) in WBC count was observed among leukaemic untreated mice when compared to aqueous *Securinega virosa* extract-treated and 5-florouracil-treated treated leukemic mice (Table 1).

Liver malondialdehyde concentration and antioxidant activities

A significant increase ($p < 0.05$) in index of oxidative stress (MDA) was observed in the liver of leukaemic-untreated mice when compared with the aqueous *Securinega virosa* extract-treated (100 mg/kg and 200 mg/kg doses) and 5-florouracil-treated groups (Table 2). Also, a significant decrease ($p < 0.05$) in catalase activities was observed in the liver of leukaemic-untreated mice (Table 2).

Histopathological studies

Photomicrograph of the liver showed mild atretic changes in the liver tissues, infiltration by RBCs, tissue necrosis, haemorrhage/fibrosis and vascular wall degeneration in the triad portal system vessels among leukaemic-untreated mice, in comparison to the extract-treated and 5-fluorouracil-treated groups (Figure 2).

Table 1: Chemotherapeutic and Chemoprotective effect of aqueous *Securinega virosa* leaf extract on hematological parameters in benzene-induced Leukaemia

Groups/ Parameters	BM Blast (%)	PB Blast (%)	WBC ($\times 10^9$)	HGB (g/dL)	RBC ($\times 10^{12}$/L)	HCT (%)	PLT ($\times 10^{12}$/L)
Control 10 mL/kg	3.00*	0.00*	4.28±0.23*	11.25±1.50*	5.46±0.99*	31.95±4.05*	624.00±33.10*
Untreated (400 mg/kg Benzene)	22.00	33.00	7.15±0.55	3.67±0.67	2.17±0.28	10.63±1.33	133.50±10.50
S. virosa (100 mg/kg)	15.00*	9.50*	5.15±0.05*	5.35±1.06*	3.75±0.79*	18.23±3.80*	794.00±122.50*
S. *virosa* (200 mg/kg)	12.00*	7.00*	3.80±0.74*	9.85±0.35*	6.45±1.23*	35.60±2.55*	814.50±155.50*
5-Fluorouracil (25 mg/kg)	13.00*	8.50*	5.05±1.05*	8.25±1.15*	5.50±2.87*	30.45±13.05*	865.0±133.50*

Values are expressed as mean ± SEM and considered significant at $p < 0.05$. * = significant difference when compared with the untreated group. Where PB = Peripheral blood, BM = Bone marrow, WBC = White blood cell, HBG = Hemoglobin, RBC = Red blood cell, PLT = Platelet, HCT = Haematocrit.

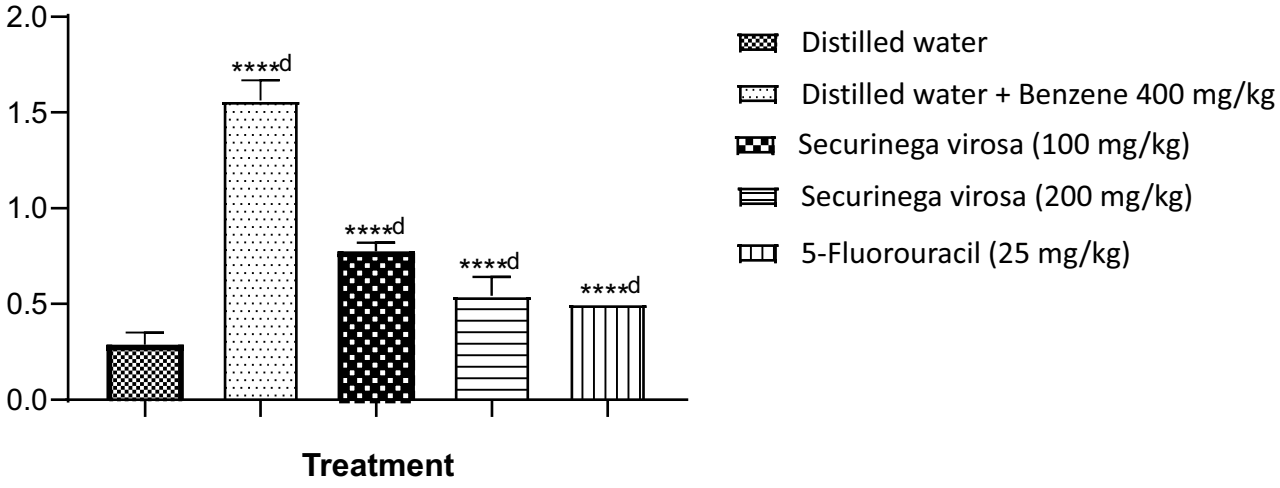
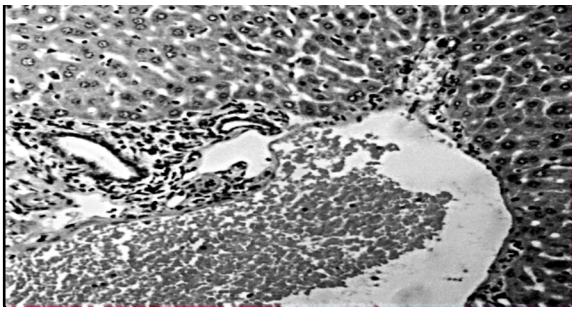


Figure 1: Percentage micro-nucleated polychromatic erythrocytes. Values were expressed as mean ± Standard Error Mean (n = 8). ****P?0.0001 vs. distilled water only (One way ANOVA by Dunnett's multiple comparison test). A significant increase ($p < 0.05$) was observed in %MNPCE value amongst leukaemic untreated mice when compared to aqueous Securinega virosa extract-treated (chemopreventive and chemotherapeutic) and 5-fluorouracil-treated groups.

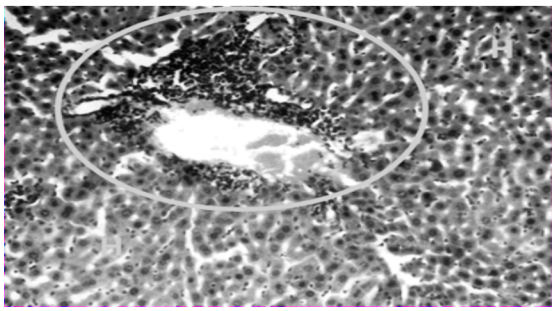
Table 2: Liver malondialdehyde concentration and antioxidant activities in benzene-induced leukaemia

Groups/ Parameters	Administered Doses	MDA (µM)	Catalase (µmoles/min/ml)
Control	10 mL/kg bw	8.12±2.40*	368.67±9.00*
Untreated	400 mg/kg bw	29.72±2.40	176.0±1.70
Securinega virosa	100 mg/kg bw	13.65±1.40*	325.56±2.70*
Securinega virosa	200 mg/kg bw	13.05±3.10*	328.67±0.00*
5-Fluorouracil	25 mg/kg bw	12.62±1.30*	336.84±6.70*

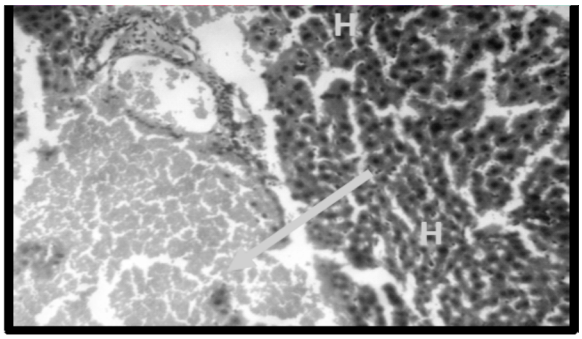
Values were expressed as mean ± SEM and considered significant at $p < 0.05$. * = significant difference when compared with the untreated group.



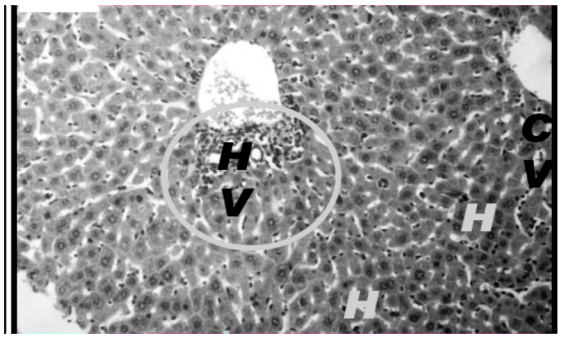
Distilled water (10 mL/kg)



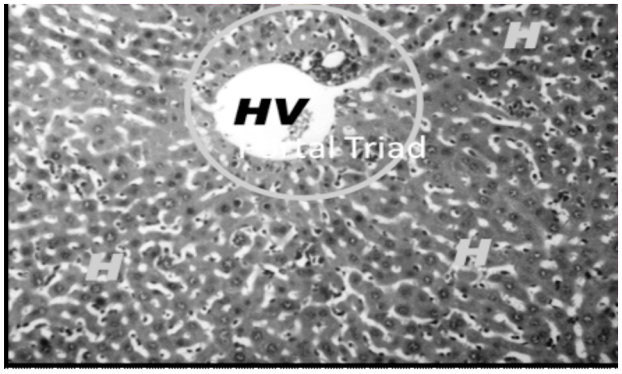
Distilled water + Benzene (400 mg/kg)



Benzene + *S virosa* (100 mg/kg)



Benzene + *S virosa* (200 mg/kg)



Benzene + 5-Fluorouracil (25 mg/kg)

Figure 2: Photomicrographs of the liver of control, leukemic untreated, and leukemic treated mice (H&E, Mag x100). Where H = Hepatocytes, HV = Hepatic vein, and arrow showed area of liver with atretic changes, infiltrated by RBCs, tissue necrosis, haemorrhage/fibrosis and vascular wall degeneration in the triad portal system vessels of leukaemic mice.

DISCUSSION

Normally, as erythroblast develops into erythrocytes in the BM, its main nucleus is extruded leaving behind a few micronuclei in the cell body. Increased numbers of micronucleated RBCs reflect chemical damage to the chromosomes or cellular mitotic apparatus. The extent of the damage is determined using the %MNPCE, which are small RNA-containing immature RBC particles consisting of acentric fragments of chromosomes or entire chromosomes present in the BM. Result from this study revealed a significant increase ($p < 0.05$) in %MNPCE value amongst leukaemic untreated mice when compared to aqueous *Securinega virosa* extract- and 5-fluorouracil-treated mice (Figure 1). This finding corroborates report from similar work where increased micronucleated cell formation was found in leukaemic Wistar rat.[26] The observed increase micronucleated cells amongst leukaemic untreated group is an indication of the genotoxic effect of benzene in bone marrow, causing chromosomal mutation, chromosomal instability, and DNA damage. There is thus compensatory induction of differentiation and multiplication or denucleation of erythroblasts present in the bone marrow and resultant increase in the numbers of normochromic erythrocytes.[27] However, the observed decrease in micronucleated cells amongst aqueous *Securinega virosa* extract-treated group indicate its ability to prevent chromosomal mutation and DNA damage which is suggestive of its anticancer activities. This might be related to its bergenin content which had been reported to inhibit proliferation and migration of cervical HeLa cancer cells; induce apoptosis and cell cycle arrest in the G0/G1 phase; and inhibit cell migration and STAT3 signaling in cervical and colorectal cancer cells.[28,29] Comparing values obtained in the group administered with 100 mg/kg *Securinega virosa* to that administered with 200 mg/kg *Securinega virosa*, a significant decrease ($p < 0.05$) in %MNPCE was observed in mice administered with 200 mg/kg *Securinega virosa* (0.5±0.05) when compared to that administered with 100 mg/kg *Securinega virosa* (0.8±0.20), indicating high activities of phytochemical bergenin in the 200 mg/kg *Securinega virosa* dose. The phytochemical bergenin present in *Securinega virosa*, exhibits some degrees of chromosomal stabilising, leukaemia-preventive and chemical-induced chromosomal damage reversal potentials.

The peripheral blood normally contains variable number of blasts ranging from zero blast to many blasts while the bone marrow usually contains ≤ 5 % blasts. Diagnosis of leukaemia requires the presence of ≥ 20% blasts or promonocytes in the peripheral blood or bone marrow. The pathognomonic disease-related morphological changes in size and shape of blood cells (WBCs, RBCs); and platelet count are of diagnostic relevance despite advancements in haematology automation and molecular technologies.[30] The percentage of WBCs constituted by blasts cells in the peripheral blood or bone marrow are of diagnostic importance and clinical relevance for the determination of leukaemia phase. Peripheral blood and bone marrow blast cells < 10% of WBCs is suggestive of chronic stable stage while blast cells in the range of 10-19 % of peripheral blood and/or nucleated bone marrow cells suggests accelerated phase. Result from this study showed the presence of blast cells in the peripheral blood and bone marrow constituting 33% and 22 % respectively (Table 1) and thus confirming establishment of leukaemia. This reflects benzene-induced haematotoxicity through its phenolic metabolites (catechol, hydroquinone) that act synergistically to cause DNA break, chromosomal damage, sister chromatid exchange, inhibition of topoisomerase II and damage to mitotic spindle. The observed clastogenic and carcinogenic effects of benzene and its metabolites is thus a reflection of topoisomerase II inhibition.[31] The administered extract however improves on the clastogenic and carcinogenic effects of benzene and its metabolites probably by stimulating topoisomerase II activities.

Leukaemia is associated with cellular abnormalities which are by no means confined to the leukocytes. In human, the WBC count in healthy situations is typically about 4,000-11,000/µL but this may be raised to 100,000-400,000 during leukaemia. Result from this study revealed a significant increase ($p < 0.05$) in WBC count among leukaemic untreated mice when compared to aqueous *Securinega virosa* extract- and 5-florouracil-treated leukemic mice (Table 1). Though values are withing normal range (2,000-10,000/µL), this finding corroborates the reported leucocytosis in leukaemia.[32,33] The observed increase in WBC count among untreated leukaemic mice could result from decreased margination of leukocytes onto vascular walls, decreased extravasation of leukocytes into tissues, compensatory proliferation of precursor cells in the marrow, on-going infection, or stimulation of the bone marrow by products of inflammatory processes. Improvement in these conditions was however brought about by the administered *Securinega virosa* extract as indicated by relatively decreased WBC count in both doses, more in the 200 mg/kg dosage.

Concerning RBC indices, a significant decrease ($p < 0.05$)

in haemoglobin concentration, haematocrit, and RBC count was observed in leukaemic untreated mice when compared to aqueous *Securinega virosa* extract- and 5 florouracil-treated leukemic mice (Table 1), corroborating the reported anaemia in leukaemia.[32] The observed decrease in RBC indices among leukaemic untreated mice is attributable to a "pre-leukaemic" bone marrow hypoplasia and haemolysis due to RBCs abnormalities phenotypically shown as poikilocytosis, spherocytosis and leptocytosis; abnormalities in osmotic fragility; and extra-corpuscular factors such as production of autoantibodies against RBCs. Non-commeasurable response of the bone marrow to increased demands for erythropoiesis consequent to accelerated haemolysis and/or bleeding disorders are also possible causes of the observed decreased haemoglobin concentration, haematocrit, and RBC count.

Normally, platelets prevent bleeding by clumping and forming plugs in blood vessel during injuries. Result from this study revealed a significant decrease ($p < 0.05$) in platelet count among leukaemic-untreated mice when compared to aqueous *Securinega virosa*- and 5-florouracil-treated leukemic mice (Table 1), though values are withing normal range (9-1,600 × 10^{12}/L). This corroborates the reported thrombocytopenia in leukaemia.[32] The observed relative thrombocytopenia among leukaemic mice is consequent to pathognomonic high concentration of immature leucocytes in the marrow which displaces the normal marrow cells, decreases platelets synthesis, and destroys platelets. Thus, as a form of extrapolation, people with leukaemia may easily become bruised (purpura); bleed into skin (petechiae) or brain; bleed from gum, nose, vagina; or develop haematuria and haematochezia. The observed increase in platelet count among the leukaemic mice administered with *Securinega virosa* extract indicate the ability of the extract to stimulate platelet synthesis and stabilise its membrane thereby preventing platelet destruction.

Oxidative stress has been linked to leukaemogenesis, as reactive oxygen species (ROS) is implicated. Antioxidant defense system protects cells from the damaging effect of endogenously-generated ROS through mitochondrial electron transport system and nicotinamide adenine dinucleotide phosphate oxidase complex during intracellular aerobic metabolism. It also regulates cell homeostasis, thereby reducing its toxic effects.[34] A linear and direct relationship exists between ROS and malondialdehyde (MDA) concentration, a marker of oxidative stress in cells. Result from this study revealed a significant increase ($p < 0.05$) in liver MDA and significant decrease ($p < 0.05$) in liver catalase activities among leukaemic-untreated mice when compared with the extract- and 5-florouracil-treated groups (Table 2). The observed increase in liver MDA concentration among leukaemic-untreated mice indicate increased ROS generation, implicating free radicals as agent of etiological importance in leukaemia. However, the observed decrease in liver catalase activities indicate its utilisation for the mopping up of benzene-induced ROS generation, thus improving cellular oxidative stress. An inverse relationship thus exists between liver MDA concentration and antioxidant status. Photomicrograph of the liver showed mild atretic changes in the liver tissues, infiltration by RBCs, tissue necrosis, haemorrhage/fibrosis and vascular wall degeneration in the triad portal system vessels among leukaemic-untreated mice (Figure 2). Improvements were however observed in liver architecture among the mice treated with aqueous *Securinega virosa* extract as shown in the photomicrograph. This confirms its anti-leukaemic activities.

CONCLUSION

Findings from this study suggest the anti-leukaemic potential of aqueous *Securinega virosa* extract as indicated by improvements in haematological parameters (%MNPCE, WBC, RBC, HCT, Hb, platelets, and blasts), markers of oxidative stress (MDA and catalase), and the general liver cyto-architecture described by well-organised portal triad and well distributed hepatocytes among the extract-treated mice. Though, biological and genetic resemblance exists between mice and human regarding development, extrapolation of the findings from this animal study should be carefully done due to human genetic diversity. The observed anti-leukaemic activities is dose related as it is higher at a dose of 200 mg/kg than 100 mg/kg. Hence by virtue of extrapolation for human use, a dose of 1.2-2.4 mg/kg (84 - 168 mg for a 70 kg man) will be appropriate as an efficient natural remedy for leukaemia in the modern era of cancer treatment.

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REFERENCES

1. Kaseb H, Madan A, Killeen RB, Hozayen S. (2023) T-Cell Prolymphocytic Leukemia. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing;.
2. Hutter JJ (2010). Childhood leukemia." *Pediatrics in review/American Academy of Pediatrics* 31(6): 234-241.
3. Bispo JAB, Pinheiro PS, Kobetz EK (2020). Epidemiology and Etiology of Leukemia and Lymphoma. *Cold Spring Harbor Perspectives Medicine* 10(6).
4. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer Journal for Clinicians* 71(3): 209-249.
5. National Cancer Institute. Cancer Stat Facts: Leukemia, 2021. Available Online. Accessed, 26/07/2021.
6. Dombret H, Gardin C (2016). An Update of Current Treatments for Adult Acute Myeloid Leukemia. *Blood* 127(1): 53-61.
7. Cosa P, Vlietinck AJ, Berghe DV, Maes L (2006). Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. *Journal Ethnopharmacology* 106: 290-302.
8. Tatematsu H, Mori M, Yang TH, Chang JJ, Lee TT, Lee KH (1991). Cytotoxic principles of Securinega virosa: virosecurininie and viroallosecurinine and related derivatives. *Journal Pharmaceutical Science* 80: 325-327.
9. Neuwinger JD. (1996) Translated from by Porter A. African ethnobotany poison and drugs. Chapman and Hall: Weinheim, 495-499.
10. Duraipandiyan V, Ayyanar M, Ignacimuthu S (2006). Antimicrobial activity of some ethno-medicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complementary Alternative Medicine*. 6: 35-41.
11. Magaji MG, Yaro AH, Musa AM, Anuka JA, Abdu-Aguye I, Hussaini IM (2012). Central depressant activities of butanol fraction of *Securinega virosa* root back in mice. *Journal of Ethnopharmacology* 141(1): 128-133.
12. Danlami U, David BM, Joyce OO, Olutayo O, Thomas SA (2013). The Antioxidant Potentials and Phytochemical Properties of the Hexane, Ethyl acetate and Ethanolic Extracts of *Securinega virosa* (Euphorbiaceae) Leaves. *Journal of Applied Pharmaceutical Science* 3(05): 131-133.
13. Magaji MG, Anuka JA, Abdu-Aguye I, Yaro AH, Hussaini IM (2008a13). Behavioural effects of the methanolic root bark extract of *Securinega virosa* in rodents. *African Journal Traditional Complement Alternative Medicine.* 5(2): 147-153.
14. Magaji MG, Anuka JA, Abdu-Aguye I, Yaro AH, Hussaini IM (2008b). Preliminary studies on anti-inflammatory and analgesic activities of *Securinega virosa* (Euphorbiaceae) in experimental animal model. *Journal of Medicinal Plants Research* 2(2): 039-044.
15. Salawu MO, Yekeen A, Nafiu MO, Oloyede HO (2019). Anti-Ulcerogenic Potential of Aqueous Extract of Securinega virosa Leaf in Indomethacin-Induced Ulcerated Rats. *Notulae Scientia Biologicae* 11(2): 196-204.
16. Soladoye MO, Amusa NA, Raji-Esan SO, Chukwuma EC, Taiwo AA (2010). Ethnobotanical Survey of Anti-Cancer Plants in Ogun State, Nigeria. *Annual Biological Research International* 1(4): 261-273.
17. Magaji MG, Ya'u J, Musa AM, Anuka JA, Abdulquadir I, Hussaini IM (2015). *Securinega virosa* (Euphorbiaceae) root bark extract inhibits glioblastoma multiforme cell survival in vitro, *African Journal of Pharmacy and Pharmacology* 9(27): 684-693.
18. Amenu J, Neglo D, Abaye D (2019). Comparative Study of the Antioxidant and Antimicrobial Activities of Compounds Isolated from Solvent Extracts of the Roots of *Securinega virosa. Journal of Biosciences and Medicines* 7: 27-41.
19. National Research Council. Guide for the care and use of laboratory animals 8th edn. *National Academy Press, Washington*, DC, 2011:41-81.
20. Lau A, Belanger CL, Winn LM (2019). In utero and acute exposure to benzene: Investigation of DNA double-strand breaks and DNA recombination in mice. *Mutation Research* 676(1-2): 74-82.
21. Liu X, Quan N. (2015). Immune Cell Isolation from Mouse Femur Bone Marrow. *Biologicae Protoc* 5(20): e1631.
22. Pearse AE. (1985) Histochemistry. Theoretical and Applied Analytical Technology. *Edinburgh: Churchill-Livingstone*, 1012-1026.
23. Iyiola S, Avwioro OG (2011). Alum haematoxylin stain for the demonstration of nuclear and extra nuclear substances. *Journal of Pharmacy and Clinical Sciences* 32: 197-199.
24. Varshney R, Kale RK (1990). Efffect of calmodulin antagonist on radiation-induced lipid peroxidation in microsomes. *International Journal of Radiation Biology* 58: 733-743.

25. Gott L (1991). A simple method for determination of serum catalase activity and revision of reference range. *Clinical Chim Acta* 196(2-3): 143-151.
26. Ola SO, Ogunkanmbi EO, Opeodu EB (2022). Chemoprotection by Kolaviron of Garcinia kola in Benzene-induced leukemogenesis in Wistar rats, *Egyptian Journal of Basic and Applied Sciences* 9: 1, 151-161.
27. Suzuki Y, Nagae Y, Li J, Sakaba H, Mozawa K, Takahashi A, Shimizu H (1989). The micronucleus test and erythropoiesis. Effects of erythropoietin and a mutagen on the ratio of polychromatic to normochromatic erythrocytes (P/N ratio). *Mutagenesis* 4(6): 420-4.
28. Gao X, Wang Y, Zhang I, Lin L, Yao Q, Xiang G (2017). Bergenin suppresses the growth of colorectal cancer cells by inhibiting PI3K/AKT/mTOR signaling pathway. *Tropical Journal of Pharmaceutical Research* 16(10): 2307-2313.
29. Shi X, Xu M, Luo K, Huang W, Yu H, Zhou T (2019). Anticancer activity of bergenin against cervical cancer cells involves apoptosis, cell cycle arrest, inhibition of cell migration and the STAT3 signalling pathway. *Experimental and Therapeutic Medicine* 17(5): 3525-3529.
30. Adewoyin AS, Nwogoh B (2014). Peripheral blood film - a review. *Annals Ibadan Postgraduate Medicine* 12(2): 71-79.
31. Chen H, Eastmond DA (1995). Topoisomerase inhibition by phenolic metabolites: a potential mechanism for benzene's clastogenic effects. *Carcinogenesis* 16(10): 2301-2307.
32. Ghosh S, Advani SH (2005). T-cell prolymphocytic leukemia- a rare case. *Indian Journal Cancer* 42(2): 104-106.
33. O'Connell KE, Mikkola AM, Stepanek AM, Vernet A, Hall CD, Sun CC, Yildirim E, Staropoli JF, Lee JT, Brown DE (2015). Practical murine hematopathology: a comparative review and implications for research. *Comparative Medicine* 65(2): 96-113.
34. Dong C, Zhang NJ, Zhang LJ (2021). Oxidative stress in leukemia and antioxidant treatment. *Chinese Medicine Journal (Engl)* 134(16): 1897-1907.