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Prophylactic Potential of Tender Coconut Water on Haematology Disorder in Benzene-Induced Lymphoid Malignancy in Wister Rats

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ABSTRACT

Background and Objective: Tender coconut water has been associated with various health and medicinal benefits. Therefore, the aim of this study was to evaluatethe prophylactic potential of tender coconut water on haematology disorder in benzene-induced lymphoid malignancy in rats in order to ascertain the potential therapeutic effect of coconut water in these disease conditions. **Materials and Methods:** Eighteen Wistar rats (170-200 g) were divided into 3 groups, namely: Negative control, positive control and phytopreventive with each group consisting of six rats. Pretreatment with 10, 20 and 30 mL kg^{-1} body weight of fresh Malayan green dwarf hybridized tender coconut water daily for 4 weeks before benzene-induced lymphoid malignancy 48 hourly for 4 weeks. Complete blood counts for haematological parameters were determined by Sysmex haematology autoanalyzer XP-300 (Kobe, Japan). **Results:** The total white blood cells, lymphocytes and platelets were significantly higher in the positive control compared to the negative control and phytopreventive group ($p<0.05$). The haematocrit value, haemoglobin value, red blood cell count and neutrophils were also significantly lower in positive control than the negative control and phytopreventive group ($p<0.01$, 0.05) while different white cell types and red blood cell indices were not significant ($p>0.05$). Induction of lymphoid malignancy in Wistar rats was successful. However, phytopreventive shows restoration of normal histology of the lymphatic organs without expression of the p53 gene and Bcl-2 except 20 and 30 mL kg^{-1} doses in the spleen that showed vacuities indicating 10 mL kg^{-1} body weight doses as preferable. **Conclusion:** This study has shown that benzene induced lymphoid malignancy is associated with the haematological disorder but was prevented with Malayan green dwarf hybridized tender coconut water with 10 mL kg^{-1} body weight daily. This has provided a unique approach to lymphoid malignant prevention.

KEYWORDS

Prophylactic, tender, coconut water, haematology disorder, benzene, lymphoid malignancies, Wistar rats

INTRODUCTION

Carcinomas, sarcomas, leukemias and lymphoid malignancy are four main groups of cancer. Cancers of epithelial origin are carcinomas that account for 90% of human malignancies. Solid tumors observed from connective tissues, such as muscle, bone, cartilage and fibrous tissue are called Sarcomas but are very rare

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in humans. Leukemia is a cancer of blood cell precursors while lymphoid malignancy is cancer arising from cells of the immune system. Leukemias and Lymphoid malignancy account for about 8% of human malignancies[1].

Haematopoiesis is a closely controlled phenomenon that yields blood cell production and any disorder can result in abnormal cell distribution and disease[2]. Blood cell lineages production take place in a haematopoietic stem cell in bone marrow in an orderly manner and any damage induced in haematopoietic stem cell (HSC) results in blood cell cancer which consequently results in altering circulating red blood cells, white blood cells and platelet counts clinically[3,4]. Presently, a complete blood count is a commonly requested investigation in the clinic that can show us an indication of anemia, cancer or toxin exposure. Hence, changes in the numbers of circulating blood cells in several lineages may reflect toxicity to haematopoietic stem cells in bone marrow[5].

Phytochemicals and phytohormones present in coconut water had shown some significant anti-aging, anti-carcinogenic and anti-thrombotic effects that contributed to the various health benefits by previous findings[6].

Benzene has been classified as a human hematological carcinogen by the International Agency for Research on Cancer (IARC)[7].

There is an increasing attempt to use coconut water in the prevention of various forms of cancer. The mechanism, dose of administration is not known and much attention has not been given to lymphoid malignancy.

Therefore, the aim of this study is to evaluate the phytopreventive effect of Malayan green dwarf hybridized immature coconut water consumption on haematology disorder in benzene-induced lymphoid malignancy in Wistar rats.

MATERIALS AND METHODS

Study area: This study was conducted at Pharmacology Department, Niger Delta University, Wilberforce, Amassoma, Bayelsa State, Nigeria from October, 2021 to April, 2022.

Animal collection: Eighteen Wistar rats were breeded in animal house of the Department of Pharmacology, Niger Delta University, Amassoma, Bayelsa State were used in this study. They were randomly selected and divided into 3 groups namely: Negative control, positive control and phytopreventive group with each group consisting of six rats.

Ethical approval: Ethical clearance was obtained from the College Health Research Ethics Committee, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State.

Research protocol: As 10, 20 and 30 mL kg^{-1} body weight of fresh Malayan green dwarf hybridized immature coconut water were given daily via orogastric intubation for four weeks for pretreatment before lymphoid malignancy was induced interperitoneally by administering 0.14 mL kg^{-1} body weight of benzene solution 48 hourly for 4 weeks. After four weeks of benzene induction, the animals were anaesthetized by placing them in a glass chamber containing cotton wool soaked in chloroform. A 25 G needle and a 5 mL syringe were then used to collect blood directly from the heart into an EDTA bottle for a complete blood count. After the animals had been sacrificed, the lymph node, bone marrow, spleen was harvested and immediately fixed in 10% formol saline solution for histology, p53 and Bcl-2 studies. Haematological parameters (complete blood count) were determined by an automated haemtology Analyzer (SYSMEX XP-300, Kobe, Japan), General tissue structure by Haematoxylin and Eosin staining techniques, p53 and Bcl-2 (Cancer Biomarkers) by Immunohistochemistry.

Fresh hybridized coconuts were harvested in the coconut farm in Opume, Ogbia local government of Bayelsa State and was identifiedas a Malayan green dwarf coconut by a plant scientist with code number UILH/001/508/2020. Qualitative and quantitative phytochemicals analysis of the coconut water was done using standard method[8].

Statistical analysis: The data obtained were analyzed by SPSS software version 22 at significant level of $p<0.05$.

RESULTS

The comparison of haematological parameters between Wistar rats without benzene-induction (negative control) and benzene-induced lymphoid malignancy in Wistar rat (positive control) as shown in Table 1. The total white blood cells count, lymphocytes and platelet count were significantly higher in benzene-induced lymphoid malignant Wistar rat (positive control) group than the negative control ($p<0.001$, 0.05, 0.01) group. The neutrophils, haematocrit value, haemoglobin value and red blood cell count were also significantly lower in the benzene-induced lymphoid malignant Wistar rat group (positive control) than the negative control group ($p<0.01$, 0.05) while different white cell types (Monocytes, Eosinphil, Basophils), mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration were not significant.

The comparison of haematological parameters between Benzene-induced lymphoid malignancy in Wistar rat (positive control) and phytopreventive (administration of 10, 20 and 30 mL kg^{-1} body weight of Malayan green dwarf hybridized immature coconut water before induction of benzene in Wistar rat) was shown in Table 2. The total white blood cell count and platelet count were significantly lower in the phytopreventive Wistar rat group than the benzene-induced lymphoid malignant Wistar rat (positive control) group ($p<0.001$, 0.01). The different white blood cells types (Monocytes, Eosinphil, Basophils), mean cell haemoglobin, haematocrit value, haemoglobin value and red blood cell count were also significantly higher in the phytopreventive group than the benzene-induced lymphoid malignant Wistar rat (positive control) ($p<0.01$, 0.05) group while lymphocytes, neutrophils, mean cell volume and mean cell haemoglobin concentration were not significant.

Comparison of haematological parameters between Wistar rat without benzene induction (negative control) group and phytopreventive group (administration of 10, 20 and 30 mL kg^{-1} body weight of malayan green dwarf hybridized immature coconut water before induction of benzene in Wistar rat) as shown in Table 3. The total white blood cells count, different white blood cells types (Monocytes, Eosinphil, Basophil, neutrophils, lymphocytes), Platelets count, haematocrit value, haemoglobin value, red blood cell counts, mean cell haemoglobin, mean cell volume and mean cell haemoglobin concentration in phytopreventive Wistar rat group were not statistically significant compared to the Wistar rat without benzene induction (negative control) ($p>0.05$).

Table 1: Comparison of haematological parameters between negative control and positive control

Haematology parameters	Negative control	Positive control	t-value	p-value
White blood cells (10^9/L)	6.10±1.93[a]	17.88±1.08[a]	-14.29	0.000
Lymphocytes (%)	76.83±12.09*	90.33±1.63*	-2.86	0.036
Neutrophils (%)	18.83±11.39*	5.67±2.42*	2.87	0.035
Mixed (%)	4.33±4.41	4.00±2.28	0.36	0.732
Platelets (10^9/L)	580.17±147.66[b]	1070.50±205.05[b]	-5.16	0.004
Haematocrit value (%)	38.33±3.98[b]	30.17±2.32[b]	4.25	0.008
Haemoglobin value (g dL^{-1})	12.72±1.38[b]	10.05±.79[b]	4.08	0.010
Red blood cells (10^{12}/L)	6.30±0.79*	5.27±0.28*	3.63	0.015
Mean cell volume (fl)	61.42±3.09	57.68±4.04	1.73	0.144
Mean cell haemoglobin (Pg)	20.35±1.02	19.12±1.41	1.70	0.151
MCHC (g dL^{-1})	33.17±0.34	33.32±0.33	-0.84	0.439

*Significant differences at $p<0.05$ level, [a]Significant differences at $p<0.001$ level, [b]Significant differences at $p<0.01$ level, Mixed: Monocytes, Eosinphil, Basophils, MCHC: Mean cell haemoglobin concentration and N = 12

Table 2: Comparison of haematological parameters between positive control group and phytopreventive group

Haematology parameters	Phytopreventive	Positive control	t-value	p-value
White blood cells (10^9/L)	7.40±2.15[a]	17.88±1.08[a]	13.90	0.000
Lymphocytes (%)	70.67±26.13	90.33±1.63	1.84	0.125
Neutrophils (%)	18.17±23.80	5.67±2.42	-1.23	0.273
Mixed (%)	9.50±3.94*	4.00±2.28*	-3.78	0.013
Platelets (10^9/L)	584.17±53.50[b]	1070.50±205.05[b]	5.94	0.002
Haematocrit value (%)	39.17±4.58[a]	30.17±2.32[a]	-7.27	0.001
Haemoglobin value (g dL^{-1})	13.15±1.51[a]	10.05±.79[a]	-8.02	0.000
Red blood cells (10^{12}/L)	6.29±0.83*	5.27±0.28*	-3.36	0.020
Mean cell volume (fl)	62.85±6.08	57.68±4.04	-2.20	0.079
Mean cell haemoglobin (Pg)	21.05±1.88*	19.12±1.41	-2.61*	0.048
MCHC (g dL^{-1})	33.53±0.40	33.32±0.33	-0.82	0.449

*Significant differences at p<0.05 level, [a]Significant differences at p<0.001 level, [b]Significant differences at p<0.01 level, Mixed: Monocytes, Eosinphil, Basophils, MCHC: Mean cell haemoglobin concentration and N = 12

Table 3: Comparison of haematological parameters between negative control group and the phytopreventive group

Haematology parameters	Negative control	Phytopreventive group	t-value	p-value
White blood cells (10^9/L)	6.10±1.93	7.40±2.12	-1.18	0.292
Lymphocytes (%)	76.83±12.09	70.67±26.13	0.47	0.658
Neutrophils (%)	18.83±11.39	18.17±23.80	0.05	0.960
Mixed (%)	4.33±4.41	9.50±3.94	-2.47	0.056
Platelets (10^9/L)	580.17±147.66	584.17±53.50	-0.07	0.950
Haematocrit value (%)	38.33±3.98	39.17±4.58	-0.33	0.757
Haemoglobin value (g dL^{-1})	12.72±1.38	13.15±1.51	-0.51	0.630
Red blood cells (10^{12}/L)	6.30±0.79	6.29±0.83	0.03	0.976
Mean cell volume (fl)	61.42±3.09	62.85±6.08	-0.57	0.593
Mean cell haemoglobin (Pg)	20.35±1.02	21.05±1.88	-0.82	0.450
MCHC (g dL^{-1})	33.17±0.34	33.53±0.40	-1.66	0.157

Mixed: Monocytes, Eosinphil, Basophils, MCHC: Mean cell haemoglobin concentration and N = 12

Table 4: Qualitative analysis of coconut water

Samples	GDHICW
Saponin	+
Tannin	+
Phenolics	+
Flavonoids	+
Terpenoids	+
Triterpene	-
Coumarin	+
Glycosides	+
Steroids	+
Aikaloid	+
Phlobatanin	-
Anthocyanin	-
Amino acids	-

+: Detected, -: Not detected, GDHICW: Green dwarf hybridized immature coconut water

Table 4 shows qualitative analysis of fresh Malayan green dwarf hybridized immature coconut water with the following bioactive phytochemical substances: Alkaloids, Tanins, Saponins, Terpernoids, Coumarins, Steroids, Flavanoids, Phenolics and Glycosides.

Quantitative phytochemical analysis of fresh Malayan green dwarf hybridized immature coconut water with a mean value of bioactive phytochemical substances: 74.42±132.75 as shown in Table 5.

The photomicrograph of the transverse section of Wistar rat's inguinal lymph node of (a) Negative control, (b) Positive control and (c) Phytopreventive group stained with Haematoxylin and Eosin (H&E)×400 magnifications was shown in Fig. 1a-c.

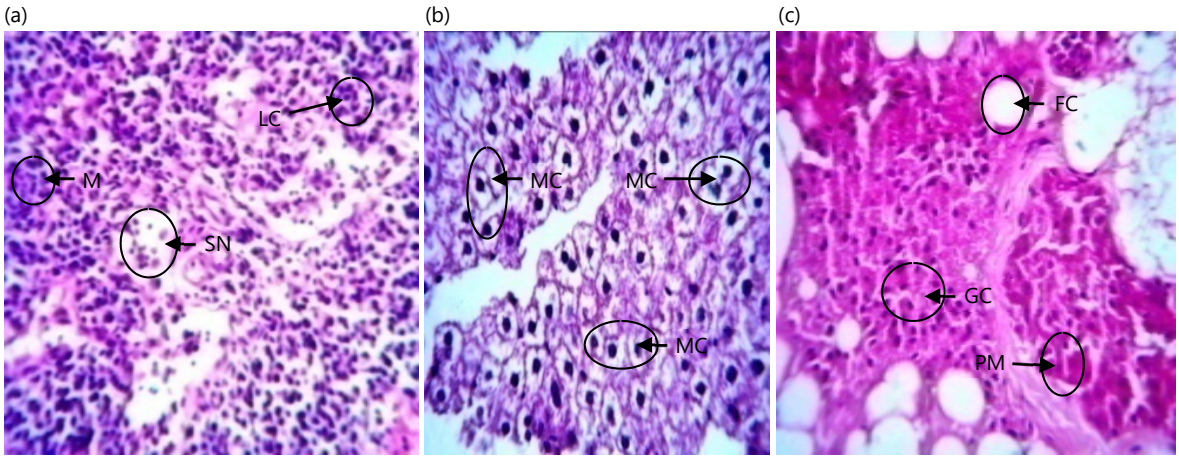

Fig. 1(a-c): Photomicrograph of the transverse section of Wistar rat's inguinal lymph node with Haematoxylin and Eosin (H&E)×400 magnifications, (a) Negative control: Normal histology of the lymph node with cortex populated with lymphocytes (LC) display memory cells (M), small non-cleaved cells (SNC), (b) Positive control: A reactive lymph node with a cortex populated with mixed populations of lymphocytes (LC) displaying classic mitotic bodies (MC) and multiple nucleations consistent with a reactive process indicating rapid dividing cells that are evidence of cancer and (c) Phytopreventive group: Restoration of normal histology of the lymph node with germinal center (GC), fats cells (FC) and perifollicular mantle (PM)

Table 5: Quantitative phytochemicals (µg/100 g) analysis of coconut water in different maturation stage

Phytochemicals	Values
Alkaloids	18.60
Tanins	3.10
Saponins	0.21
Terpernoids	9.70
Coumarins	27.40
Steroids	149.80
Flavanoids	48.00
Phenolics	406.00
Glycosides	7.00
N	9
Mean±SD	74.42±132.75

The photomicrograph of the transverse section of Wistar rat's spleen of (a) Negative control, (b) Positive control and (c) Phytopreventive group stained with Haematoxylin and Eosin (H&E)×400 magnification were shown in Fig. 2a, b, c_1, c_2 and c_3.

The photomicrograph of the transverse section of Wistar rat's bone of (a) Negative control, (b) Positive control and (c) Phytopreventive group stained with Haematoxylin and Eosin (H&E)×400 magnifications was shown in Fig. 3a-c.

Figure 4 showed p53 gene immunohistochemistry of Wistar rat's inguinal lymph node of (a) Negative control, (b) Positive control and (c) Phytopreventive group.

The Bcl-2 immunohistochemistry of Wistar rat's inguinal lymph node from group of (a) Negative control, (b) Positive control and (c) Phytopreventive group was shown in Fig. 5a-c.

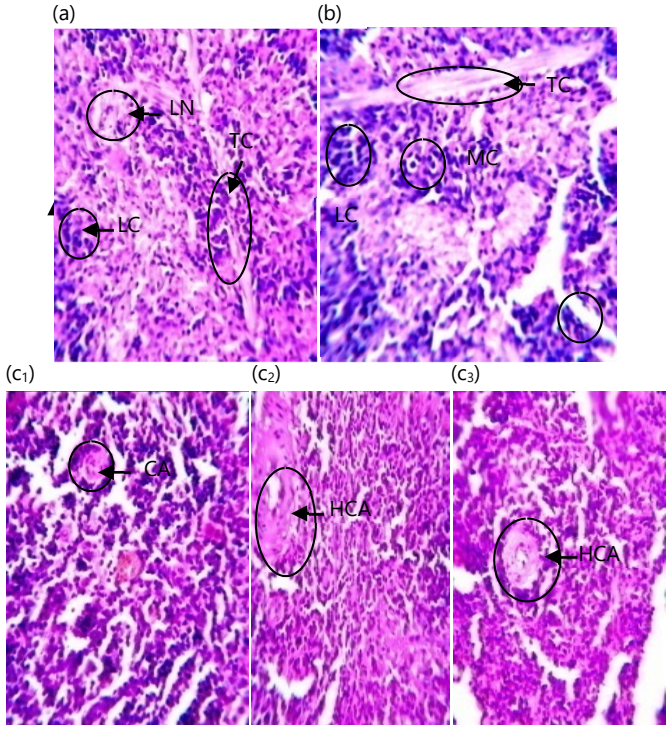

Fig. 2(a-c): Photomicrograph of the transverse section of Wistar rat's spleen stained with Haematoxylin and Eosin (H&E)×400 magnifications, (a) Negative control: Normal histology of the spleen with the tissue parenchyma contains lymphocytes population, lymphatic nodule (LN), trabecula (TC), central arterioles (CA) and germinal centre (GM), (b) Positive control: Reactive spleen with the tissue parenchyma with lymphocytes population, enlarged trabeculae (TC) and mitotic bodies (MC) consistent with reactive spleen indicating rapid dividing cells, which is evidence of cancer, (c_1) Restoration of normal histology of the spleen with the tissue parenchyma containing lymphocytes population (LC), lymphatic nodule (LN), central arterioles (CA), trabecula (TC) and trabeculae artery (TA) and (c_{2-3}) Phytopreventive: 20 and 30 mL kg^{-1} doses shows the tissue parenchyma with lymphocytes population, lymphatic nodule and hypertrophic central arterioles indicating vacuities

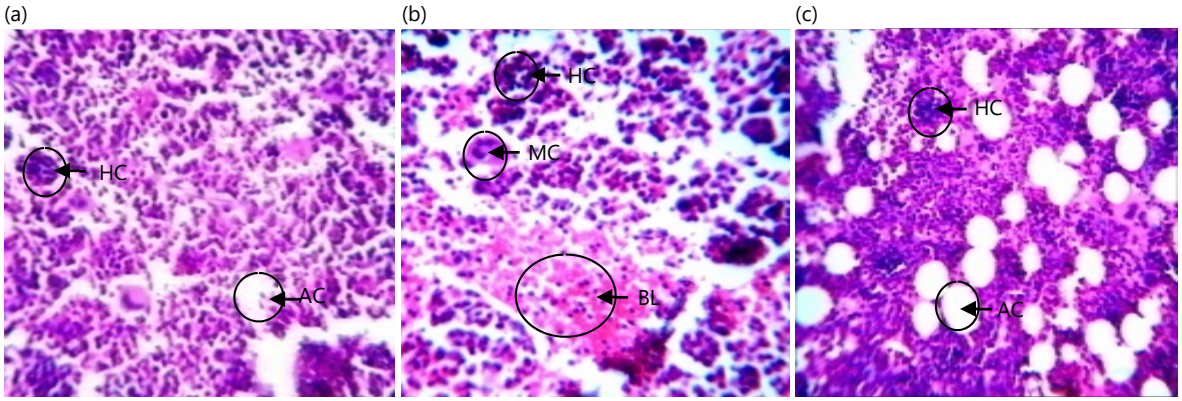

Fig. 3(a-c): Photomicrograph of the transverse section of Wistar rat's bone marrow stained with Haematoxylin and Eosin (H&E)×400 magnifications, (a) Negative control: Normal histology of the bone marrow with haematopoietic cells (HC) and adipocytes (AC), (b) Positive control: Reactive bone marrow with haematopoietic cells (HC), invasion of bone marrow by blasts (BL), mitotic bodies (MC) consistent with reactive bone marrow and (c) Phytopreventive group: Restoration of normal histology of the bone marrow with haematopoietic cells (HC) and adipocytes (AC)

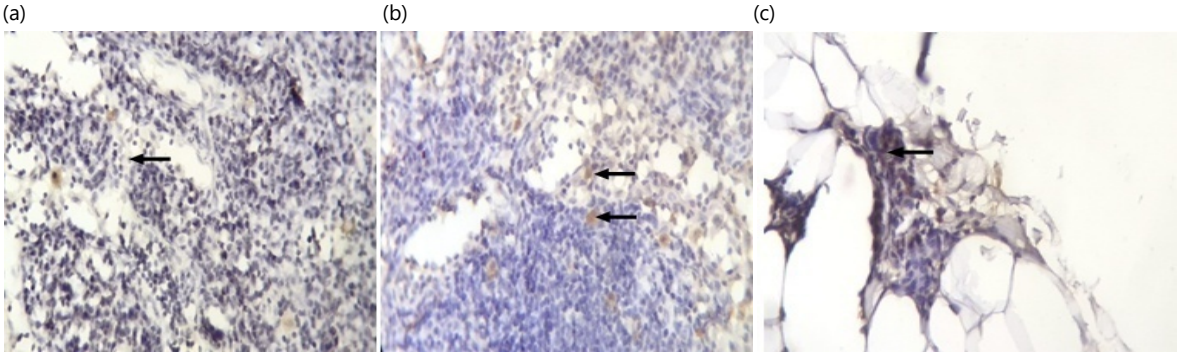

Fig. 4(a-c): p53 gene immunohistochemistry of Wistar rat's inguinal lymph node for (a) Negative control: Negative nuclear immunohistochemistry reaction (black arrow), (b) Positive control: Selective positive nuclear immunohistochemistry reaction (black arrow) and (c) Phytopreventive group: Restoration of normal immunohistochemistry of the inguinal lymph node with negative nuclear immunohistochemistry reaction (black arrow)

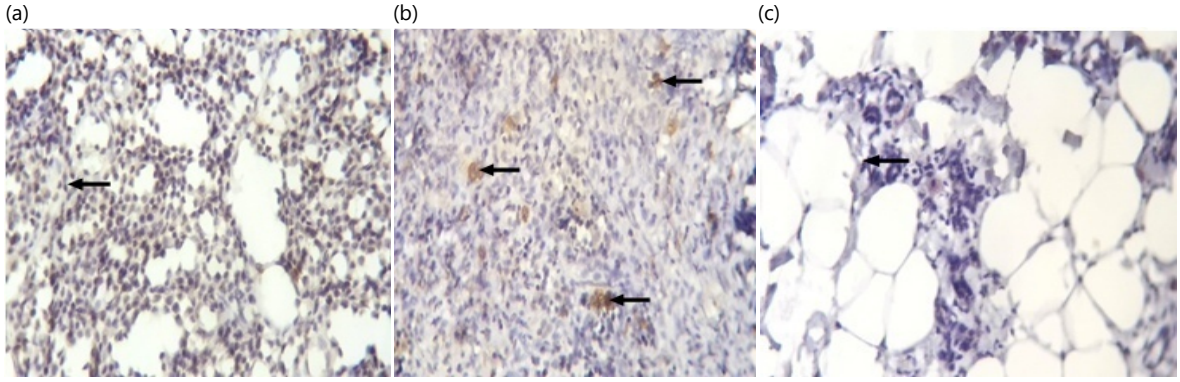

Fig. 5(a-c): Bcl-2 immunohistochemistry of Wistar rat's inguinal lymph node for (a) Negative control: Negative cytoplasmic staining pattern (black arrow), (b) Positive control: Positive cytoplasmic staining pattern (black arrow) and (c) Phytopreventive group: Restoration of normal immunohistochemistry of the inguinal lymph node with a negative cytoplasmic staining pattern (black arrow)

DISCUSSION

Leucocytosis, thrombocytosis and anaemia associated with benzene-induced lymphoid malignancy were observed to have been prevented in Wistar rats treated with 10, 20 and 30 mL kg^{-1} body weight of Malayan green dwarf hybridized immature coconut water before exposure to benzene inducing agent showed significantly lower in the total white blood cell count, platelets count and significantly higher in haematocrit value, haemoglobin, red blood cell count of Wstar rats in phytopreventive group compared to positive control.

The effect of benzene on haematological parameters could be due to its toxic effect on haematopoietic cells in the bone marrow. This was in agreement with the study that showed that blood is the most essential tissue in the body that alters metabolic activities therefore, the common dependable pointer of the toxic effect of drugs and heavy metals is an alteration in blood parameters and indices[9]. The phytotherapeutic and phytopreventive effect observed in the haematological parameters in this study could be due to Malayan green dwarf hybridized immature coconut water bioactive phytochemical and phytohormones substances such as Phenolic compounds, Alkaloids, Saponins, Glycosides, Triterpenoids

and Gibberellin that possess anticancer properties. Phenolic Compounds express better anticancer activity base on the number of hydroxylic groups found in the compounds[10,11]. Alkaloids, Saponins, glycosides anticancer mechanism activities are by blocking cell proliferation by inducing cell cycle arrest at the G1 or G2/M phases[12-15]. Triterpenoids block initiation and promotion of carcinogenesis, create neoplasm cell differentiation and apoptosis[16-19]. This finding is supported by the report of a previous works conducted by various researchers in which exposure to benzene resulted to thrombocytosis, leucocytosis and anaemia but was ameliorated by natural plant extracts that possess such phytochemical bioactive substances[20-23].

Malayan green dwarf hybridized immature (HI) coconut water with the following phytochemical bioactive substances: Alkaloids, Tanins, Saponins, Terpernoids, Coumarins, Steroids, Flavanoids, Phenolics, Glycosides and Phytohormones, Cytokinins, Gibberellins, Ascorbic acid, Apigenin, Rutin, Ferulic Acid was observed to be more beneficial. This could be attributed to the fact that Malayan green dwarf hybridized immature (HI) coconut water has the highest content of phytochemicals and phytohormones. This was inline other previous study on Quantification of Cytokinins in coconut water from different maturation stages of Malaysia's coconut (*Cocos nucifera* L.) varieties that show that Malayan green dwarf hybridized immature (HI) coconut water has the highest content of phytohormones[24].

Consequently, in this study, the histology reports of inguinal lymph node, spleen, bone marrow in the negative control (Wistar rat without induction of benzene) shows the cortex populated with lymphocytes, displaying memory cells, small non cleaved cell consistent with normal histology of the lymph node as seen in lymph node, the tissue parenchyma with lymphocytes population, lymphatic nodule, trabecule, centralarterioles and germinal center consistent with normal histology of the spleen as observed in spleen and haematopoietic cells, adipocytes as seen in the bone marrow without expression of p53 and Bcl-2 cancer biomarkers.

Furthermore, this study demonstrated the successful induction of lymphoid malignancy in Wistar rats Interperitoneally with 0.14 mL kg^{-1} body weight of benzene 48 hourly for 4 weeks as seen in the histology reports in the inguinal lymph node, spleen and bone marrow in the positive control group (benzene induced) showing the cortex populated with mixed populations of lymphocytes in lymph node, parenchyma with lymphocytes population, enlarged trabeculae in the spleen, haematopoietic cells, adipocytes, megakaryocytes, the invasion of bone marrow by the blasts and all the tissues displaying classic mitotic bodies and multiple nucleations consistent with reactive process indicating rapid dividing cells which is evidence of cancer with overexpression of p53 and Bcl-2 cancer biomarkers. Benzene induced lymphoid malignancy was inconsistent with a previous study where benzene was used to induce leukaemia[22].

Overexpression of p53 was in agreement with another study in which it was found that p53 overexpression might be an indicator of cancer that is aggressive and poor prognosis[25].

Overexpression of Bcl-2 was in agreement with previous studies in which it was found that overexpression of Bcl-2 is a feature of lymphoid malignancy[26] and also Bcl-2 expression by immunohistochemistry was replicable and the results were conformable with those of flow cytometry and immunoblotting[27].

However, the histology reports of the inguinallymph node, spleen, bone marrow of phytopreventive showed restoration of normal histology of the lymph node with small non-cleaved cells, lymphoid follicle, lymphocytes, germinal center, fats cells, perifollicular mantle, memory cells and post capillary venule, restoration of normal histology of the spleen with the tissue parenchyma containing lymphocytes population, lymphatic nodule, central arterioles, trabeculae artery except 20 and 30 mL kg^{-1} doses with hypertrophic central arterioles indicating Vacuities. This indicated that 10 mL kg^{-1} doses were preferable,

Restoration of normal histology of the bone marrow with haematopoietic cells and adipocytes without expression of the p53 gene and Bcl-2 cancer biomarkers. The restoration of normal histology of the lymph node, spleen and bone marrow by phytopreventive could be that bioactive phytochemical and phytohormone substances in Malayan green dwarf hybridized immature coconut water needed a specific dose to be effective. This was consistent with a study on synergistic and ameliorative effect of honey and coconut water on crude oil induced toxicity in rats where doses of 10 mL kg^{-1} body weight daily was reported[28]. This imply that Malayan green dwarf hybridized tender coconut water has prophylactic potential on haematology disorder in benzene-induced lymphoid malignancy in Wistar rats with a recommended dose of 10 mL kg^{-1} body weight daily. This is critical information for preventive medicine for human clinical trials. Further study can be done to evaluate the phytotherapeutic potential of Malayan green dwarf hybridized tender coconut water and other species of coconut water can be incorporated.

CONCLUSION

This study reviews the phyto-preventive properties of Malayan green dwarf hybridized tender coconut water. The recommended doses of Malayan green dwarf hybridized tender coconut water for maximum effect on haematology disorder associated with lymphoid malignancy were 10 mL kg^{-1} body weight daily for phyto-preventive. This study also provides suggestive evidence that benzene can induce lymphoid malignancy.

SIGNIFICANCE STATEMENT

This study discovers prophylactic potential of Malayan green dwarf hybridized immature (HI) coconut water on haematological disorder associated lymphoid malignancy showing critical information for preventive medicine with doses of 10 mL kg^{-1} body weight. This study will help the researcher to uncover the critical area of the prophylactic potential of coconut water phytochemicals on malignant conditions that many researchers were not able to explore. Thus, a new theory on these coconut water phytochemicals may be arrived at.

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REFERENCES

1. Cooper, G.M., 2000. The Cell: A Molecular Approach. 2nd Edn., ASM Press, Washington, DC, ISBN: 9780878931064, Pages: 689.
2. Stein, S.J. and A.S. Baldwin, 2013. Deletion of the NF-κB subunit p65/RelA in the hematopoietic compartment leads to defects in hematopoietic stem cell function. Blood, 121: 5015-5024.
3. Passegué, E., C.H.M. Jamieson, L.E. Ailles and I.L. Weissman, 2003. Normal and leukemic hematopoiesis: Are leukemias a stem cell disorder or a reacquisition of stem cell characteristics? Proc. Nat. Acad. Sci. U.S.A., 100: 11842-11849.
4. Schiffman, C., C.M. McHale, A.E. Hubbard, L. Zhang and R. Thomas *et al*., 2018. Identification of gene expression predictors of occupational benzene exposure. PLoS ONE, Vol. 13. 10.1371/journal.pone.0205427.
5. Wick, J., 2013. Bone marrow: The workhorse organ. Consultant Pharm., 28: 16-22.
6. Rattan, S.I.S. and L. Sodagam, 2005. Gerontomodulatory and youth-preserving effects of zeatin on human skin fibroblasts undergoing aging *in vitro*. Rejuvenation Res., 8: 46-57.
7. Snyder, R., 2012. Leukemia and benzene. Int. J. Environ. Res. Public Health, 9: 2875-2893.
8. Ojha, S.B., S. Roy, S. Das and G. Dhangadamajhi, 2019. Phytochemicals screening, phenolic estimation and evaluation for anti-oxidant, anti-inflammatory and anti-microbial activities of sequentially soxhlet extracted coconut testa. Food Nutr. Sci., 10: 900-922.

9. Kim, Y., A. Jaja-Chimedza, D. Merrill, O. Mendes and I. Raskin, 2018. A 14-day repeated-dose oral toxicological evaluation of an isothiocyanate-enriched hydro-alcoholic extract from *Moringa oleifera* Lam. seeds in rats. Toxicol. Rep., 5: 418-426.
10. Lee, Y.J., P.H. Liao, W.K. Chen and C.Y. Yang, 2000. Preferential cytotoxicity of caffeic acid phenethyl ester analogues on oral cancer cells. Cancer Lett., 153: 51-56.
11. Chen, M., H. Meng, Y. Zhao, F. Chen and S. Yu, 2015. Antioxidant and *in vitro* anticancer activities of phenolics isolated from sugar beet molasses. BMC Complementary Altern. Med., Vol. 15. 10.1186/s12906-015-0847-5.
12. Qi, J., A.H.M. Zulfiker, C. Li, D. Good and M.Q. Wei, 2018. The development of toad toxins as potential therapeutic agents. Toxins, Vol. 10. 10.3390/toxins10080336.
13. Tin, M.M.Y., C.H. Cho, K. Chan, A.E. James and J.K.S. Ko, 2007. *Astragalus* saponins induce growth inhibition and apoptosis in human colon cancer cells and tumor xenograft. Carcinogenesis, 28: 1347-1355.
14. Jiang, Y., Y. Zhang, J. Luan, H. Duan, F. Zhang, K. Yagasaki and G. Zhang, 2010. Effects of bufalin on the proliferation of human lung cancer cells and its molecular mechanisms of action. Cytotechnology, 62: 573-583.
15. Burgeiro, A., C. Gajate, E.L.H. Dakir, J.A. Villa-Pulgarín, P.J. Oliveira and F. Mollinedo, 2011. Involvement of mitochondrial and B-RAF/ERK signaling pathways in berberine-induced apoptosis in human melanoma cells. Anti-Cancer Drugs, 22: 507-518.
16. Panucci, R.K.I., A. Mellitto, C.R. Oliveira, W. de Mello Marin and C. Bincoletto, 2016. *In vitro* study of anti-leukemic potential of ursolic acid in jurkat cell line. J. Clin. Exp. Oncol., Vol. 5. 10.4172/2324-9110.1000161.
17. Liu, J., 2005. Oleanolic acid and ursolic acid: Research perspectives. J. Ethnopharmacol., 100: 92-94.
18. Liby, K.T., M.M. Yore and M.B. Sporn, 2007. Triterpenoids and rexinoids as multifunctional agents for the prevention and treatment of cancer. Nat. Rev. Cancer, 7: 357-369.
19. Patlolla, J.M.R. and C.V. Rao, 2012. Triterpenoids for cancer prevention and treatment: Current status and future prospects. Curr. Pharm. Biotechnol., 13: 147-155.
20. Ebrahimi, A., A. Atashi, M. Soleimani, M. Mashhadikhan, A. Barahimi and A. Maghari, 2018. Anti-invasive and antiproliferative effects of *Pleurotus ostreatus* extract on acute leukemia cell lines. J. Basic Clin. Physiol. Pharmacol., 29: 95-102.
21. Akanni, E.O., A.L. Adedeji, O.T. Adedosu, O.I. Olaniran and J.K. Oloke, 2014. Chemopreventive and anti-leukemic effects of ethanol extracts of *Moringa oleifera* leaves on Wistar rats bearing benzene induced leukemia. Curr. Pharm. Biotechnol., 15: 563-568.
22. Olufemi, A.E., F. Ayodeji, A.R. Adekemi, B.E. Oluseyi, A.F. Ajoke, A.O. Aminat and L.O. Idris, 2017. African polyherbal formulation possesses chemopreventive and chemotherapeutic effects on benzene- induced leukemia in Wistar rats. Annu. Res. Rev. Biol., Vol. 16. 10.9734/ARRB/2017/34773.
23. Olufemi, A.E., A.O.A. Terry and O.J. Kola, 2012. Anti-leukemic and immunomodulatory effects of fungal metabolites of *Pleurotus pulmonarius* and *Pleurotus ostreatus* on benzene-induced leukemia in Wister rats. Korean J. Hematol., 47: 67-73.
24. Lazim, M.I.M., N.A. Badruzaman, K.S. Peng and K. Long, 2015. Quantification of cytokinins in coconut water from different maturation stages of Malaysia's coconut (*Cocos nucifera* L.) varieties. J. Food Process Technol., Vol. 6. 10.4172/2157-7110.1000515.
25. Abdul-Razaq, H.K., M.H. Alhawaz and N.H.J. Al-Echrish, 2016. Evaluation of the p53 gene expression in breast cancer in respect to age, grade, stage and lymph nodes. Basrah J. Surg., 22: 17-25.
26. Roberts, A.W., M.S. Davids, J.M. Pagel, B.S. Kahl and S.D. Puvvada *et al*., 2016. Targeting BCL2 with venetoclax in relapsed chronic lymphocytic leukemia. N. Engl. J. Med., 374: 311-322.
27. Marschitz, I., I. Tinhofer, A. Hittmair, A. Egle, M. Kos and R. Greil, 2000. Analysis of Bcl-2 protein expression in chronic lymphocytic leukemia: A comparison of three semiquantitation techniques. Am. J. Clin. Pathol., 113: 219-229.
28. Akintola, A.O., B.D. Kehinde, J.O. Fakunle and A.F. Ajayi, 2018. Synergistic and ameliorative effect of honey and coconut water on crude oil induced toxicity in rats. Res. J. Environ. Toxicol., 12: 24-33.