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ORIGINAL ARTICLE

Modulatory Effects of Selected Medicinal Plant Extracts on Benzene-Induced Haematopoietic Dysfunction in Wistar Rats

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ABSTRACT

Benzene exposure has been linked to haematopoietic toxicity via generation of oxidative stress and DNA damage and breakdown of bone marrow function. Medicinal plants containing bioactive phytochemicals may offer protective effects against chemical-induced haematological alterations. This study evaluated the modulatory effects of selected medicinal plant extracts on benzene-induced haematopoietic dysfunction in Wistar rats. Forty-two male Wistar rats, weighng 80 -120g were used. They were randomly divided into 7 groups (n= 6). Group A (Normal control) received only normal saline, Group B (benzene group), Group C (benzene + *Azadirachta indica* , *Psorospermum febrifugum*, *Ricinus communis* and *Rosy periwinkle* from Groups C1-C4 respectively) while group D (benzene+ 5-fluorouracil). The haematological effects of animals were examined in terms of the change in parameters of full blood count and peripheral blood morphology. The results indicate **b**enzene exposure produced significant alterations in haematological indices compared with the control group, including changes in white blood cell count, haemoglobin concentration, packed cell volume, and platelet parameters ($p < 0.05$). Treatment with the plant extracts partially restored altered haematological parameters, with some extracts showing effects comparable to the 5-fluorouracil-treated group. Selected medicinal plant extracts demonstrated potential modulatory effects against benzene-induced haematopoietic dysfunction in Wistar rats. These findings support further investigation of their phytochemical constituents and mechanisms of action.

Keywords: Benzene toxicity; Haematopoietic dysfunction; Medicinal plant extracts; Wistar rats; Haematological parameters.

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1.0 INTRODUCTION

Leukaemia is a diverse group of haematological cancers that originate from unchecked growth and differentiation deficits in haematopoietic stem and progenitor cells, and the consequential failure of blood cell production, which leads to progressively compromised bone marrow function (Bispo et al., 2020; Khy et al., 2022). This failure is generally characterised clinically by anaemia, thrombocytopenia and immune dysfunction and is responsible for a significant proportion of mortality associated with the disease (Bispo et al., 2020; Khy et al., 2022). Even with the improvement in chemotherapy regimens and targeted therapy, there is still a heavy disease burden due to toxicity, high costs and relatively poor access to chemotherapeutic treatment, emphasising the need for the development and investigation of safe complementary approaches. Benzene is an established environmental and occupational haematotoxin with bone marrow toxicity and haematological pathologies, including acute myeloid leukaemia and myelodysplastic syndromes (Huff, 2007; Mc Hale et al., 2012; Huang et al., 2022). Benzene continues to be an important public health concern relative to occupational and environmental exposure to petroleum related substances. Well documented haematological and systemic toxic responses are evident among benzene exposed subjects (Cordiano et al., 2022). Recent molecular research has suggested oxidative stress, regulation of inflammation and pathways of haematopoietic cells as potential mechanisms of benzene haematotoxicity (He et al., 2024; Zhang et al., 2024; Wang et al., 2024). The haematotoxicity of benzene is mediated through hepatic metabolites, including hydroquinone, catechol, and benzoquinone, which compromise RBC and BM cellular function through induction of Oxidative stress, DNA-damage, inflammatory pathways, and disruptions to normal hematopoietic cellular processes. Such pathways consequently impair the viability, proliferation, and differentiation of haematopoietic progenitor cells, expressed as erythrocytopenia, leukopenia and thrombocytopenia (Huff, 2007; Mc Hale et al., 2012). As a result, experimental benzene exposure models are usually employed to explore chemical-initiated haematopoietic disruption and abnormalities associated with preleukemoid development; however molecular identification or cellular histoprofiling of malignant transdifferentiation is often required to validate lesion malignancy. Haematological indices (including white blood cell count, haemoglobin concentration, PCV and platelet count) are also useful in assessing the physiological functions of the bone marrow and the blood system as a whole (Percival et al., 2017). Likewise, cellular morphology and abnormal features in the blood periphery can be assessed through examination of a blood film (Kumar, 2018; Didehban et al., 2024). Medicinal plants have received increased research focus because of attributes such as antioxidant, anti-inflammatory, immunomodulatory and anticancer activity, with phytochemicals such as alkaloids, flavonoids, phenolic compounds, terpenoids identified as having potential implications in parameters such as oxidative injury reduction and the modulation of pathways responsible for cell damage and inflammation (Palhares et al., 2015; Kooti et al., 2017).

Azadirachta indica has been reported to have antioxidant and immunomodulatory activity; *Rosy periwinkle* (*Catharanthus roseus*) has been shown to have compounds (vinca alkaloids) such as vincristine and vinblastine with anti-cancer activity (Kooti et al., 2017). *Ricinus communis* and *Psorospermum febrifugum* have constituents which have reported antioxidant and pharmacological activities (Rasool, 2012; Otu-Boakye et al., 2024). Despite these mentioned biological activities, there is paucity of knowledge on how the different medicinal plant extracts would affect benzene-induced haematological disorders. Thus in this paper, the modulatory effects of some medicinal plant extracts (*Azadirachta indica* , *Psorospermum febrifugum*, *Ricinus communis*, and *Rosy periwinkle*) in Wister rats on benzene-induced haematotoxicity were studied through the examination of some peripheral blood parameters and blood film patterns.

2.0 MATERIALS AND METHODS

2.1 Collection, identification and preparation of plant materials

The medicinal plants used in this study included *Azadirachta indica* , *Psorospermum febrifugum*, *Ricinus communis*, and *Catharanthus roseus* (*Rosy periwinkle*). The plant materials were collected from the vegetation around the College of Basic Medical Sciences, Ladoke Akintola University of Technology, Osogbo, Osun State, Nigeria. The plants were authenticated at the Department of Plant Science, Obafemi Awolowo University, Ile-Ife, Nigeria. The plant materials were washed, air-dried and ground prior to extraction.

2.2 Preparation of plant extracts

For the aqueous extracts, 200 g of each powdered plant material was extracted using 500 mL of distilled water. The mixture was stirred for two h at room temperature and filtered using Whatman filter paper. The filtrates were concentrated using a rotary evaporator at 40°C to obtain semi-solid extracts. The *Ricinus communis* extract was prepared separately using nhexane extraction through Soxhlet extraction. Different extraction methods were based on the traditional preparation and phytochemical properties of individual plant materials. The extract was concentrated using a rotary evaporator at 40°C and prepared for administration. The prepared extracts were stored appropriately until administration.

2.3 Experimental animals and housing conditions

Male Wistar rats weighing 80–120 g were obtained from the animal house of Ladoke Akintola University of Technology (LAUTECH), Osogbo, Nigeria. The animals were acclimatised for seven days before commencement of the experiment. They were housed under standard laboratory conditions with a 12h light/dark cycle, temperature maintained at 28 ± 2°C, and provided with standard rat feed and water ad libitum throughout the experimental period.

2.4 Experimental design

Forty-two (42) Wistar rats were randomly allocated into four experimental groups (A-D) (n=6), with Group C further subdivided into C1–C4, comprising six rats per subgroup, as follows:

Group A (*Control group)*: Normal Saline (0.5 mL/kg orally for 4 weeks).
Group B *(Benzene-induced group)*: Benzene Chromasolv (0.2 mL, 1:10 dilution in water/2-propanol via daily tail vein injection for 4 weeks).
Group C *(Plant extract treatment groups)*: Aqueous extracts of *Azadirachta indica* (C1), *Psorospernum febrifugum* (C2), *Ricinus communis* (C3), and *Rosy periwinkle* (C4) at 10 mg/kg body weight, respectively.
Group D *(Standard treatment group)*: 5-Fluorouracil (5 mg/kg body weight)

The dose of 5-Fluorouracil used in this study is consistent with standard protocols for inducing chemotherapy-related effects in animal models of leukemia as reported by Fleischmann et al. (2021).

2.5 Benzene-induced haematopoietic dysfunction model

Benzene Chromasolv (Sigma-Aldrich, lot #STBD7135Y, >99.9%) was diluted 1:10 with water for injection (benzene:water, 1:9 v/v), and 0.2 mL of the diluted solution was administered through the tail vein every 48 h for four weeks to induce haematopoietic dysfunction (Akanni et al., 2017).

2.6 Haematological analysis

At the end of the experimental period, blood samples were collected for haematological evaluation. The analysed parameters included: WBC, RBC, Hb, PCV, Platelet count and differential leukocyte count. Complete blood count analysis was performed using Sysmex KX-21N auto-analyser. Peripheral blood films were prepared, stained with Leishman staining technique and examined microscopically to assess morphological changes associated with benzene-induced haematopoietic alterations.

2.7 Ethical approval

This experimental study was designed to assess modulatory potentials of some specified medicinal plants extracts against benzene induced haematopoietic abnormalities in Wistar rats. Experimental protocols and procedures adhered to established guidelines for the care and use of laboratory animals. Ethics approval was obtained from the Osun State Health Research Ethics Committee (OSHREC/PRS/569T/156), Osun State Ministry of Health Nigeria.

2.8 Statistical analysis

Data obtained from the study were analysed using IBM Statistical Package for the Social Sciences (SPSS) version 25.0. Results were presented as mean ± standard error of mean (SEM). Differences among experimental groups were analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Statistical significance was considered at $p < 0.05$.

3.0 RESULTS

3.1 Effect of Benzene Exposure and Plant Extract Treatment on Body Weight Changes

Changes in body weight following benzene exposure and administration of selected medicinal plant extracts are presented in Figure 1. The benzene-induced group showed a significant reduction in final body weight compared with the control group ($p < 0.05$). Similarly, the 5-fluorouracil-treated group exhibited a significant reduction in final body weight compared with the control group ($p < 0.05$). There was no statistically significant difference in final body weight between the plant extract treated groups (*Azadirachta indica* , *Psorospermum febrifugum*, *Ricinus communis*, and *Catharanthus roseus*) and the benzene induced group.

3.2 Effect of Selected Medicinal Plant Extracts on Haematological Parameters in Benzene-Induced Haematopoietic Dysfunction

The effects of benzene exposure and plant extract administration on haematological parameters are presented in Table 1. The benzene-induced group showed a significant increase in total white blood cell (WBC) count (25.3 ± 3.8 ×10³/µL) compared with the control group (6.0 ± 1.2 ×10³/µL) ($p < 0.05$). Administration of *A. indica*, *P. febrifugum*, *R. communis*, and *C. roseus* reduced WBC counts compared with the benzene-induced group, with values of 14.1 ± 2.4, 12.4 ± 2.1, 13.2 ± 2.5, and 14.0 ± 2.0 ×10³/µL, respectively. Haemoglobin concentration and packed cell volume were significantly reduced in the benzene-induced group compared with the control group ($p < 0.05$). The benzene-induced group recorded haemoglobin and packed cell volume values of 8.5 ± 1.1 g/dL and 28 ± 5%, respectively, compared with 13.5 ± 0.5 g/dL and 40 ± 4% in the control group. Treatment with plant extracts increased haemoglobin concentration and packed cell volume compared with the benzene-induced group, with the highest haemoglobin value observed in the *P. febrifugum*-treated group (11.8 ± 1.2 g/dL). Platelet count was significantly reduced in the benzene-induced group (110 ± 20 ×10³/µL) compared with the control group (320 ± 25 ×10³/µL). Plant extract administration increased platelet counts compared with benzene exposure alone, with the highest value observed in the *R. communis*-treated group (250 ± 22 ×10³/µL).

3.3 Effect of Plant Extract Administration on Differential Leukocyte Profiles Following Benzene Exposure

Differential leukocyte profiles are presented in Table 2**.** The benzene-induced group showed a significant reduction in neutrophil percentage (35 ± 6%) and an increase in lymphocyte percentage (50 ± 7%) compared with the control group (neutrophils: 60 ± 5%; lymphocytes: 30 ± 4%) ($p < 0.05$). Treatment with the plant extracts increased neutrophil percentage and reduced lymphocyte percentage compared with the benzene-induced group. The *A. indica*-treated group recorded neutrophil and lymphocyte values of 52 ± 4% and 38 ± 5%, respectively, while the *P. febrifugum*-treated group recorded 50 ± 5% and 40 ± 6%, respectively. The 5-

fluorouracil-treated group showed neutrophil and lymphocyte values of 55 ± 4% and 35 ± 5%, respectively.

3.4 Morphological Assessment of Peripheral Blood Cells Following Benzene Exposure and Treatment

Representative peripheral blood films from the experimental groups are presented in Figure 2. The control group showed normal peripheral blood cell morphology, whereas the benzene-induced group demonstrated increased leukocyte density and altered cellular morphology. The plant extract-treated groups showed reduced leukocyte density and comparatively improved cellular morphology relative to the benzene-induced group.

Table **1**. Effect of selected medicinal plant extracts on haematological parameters in benzene-induced haematopoietic dysfunction in Wistar rats

Group	WBC (x10³/µL)	HGB (g/dL)	HCT (%)	Platelet Count (x10³/µL)	F-value	p-value
Control (A)	6.00 ± 1.20	13.52 ± 0.53	40.0 ± 4.0	320.0 ± 25.0	NS	N.S
Benzene-induced (B)	25.30 ± 3.80	8.51 ± 1.10	28.0 ± 5.0	110.0 ± 20.0	5.20	<0.001*
Azadirachta indica (C1)	14.10 ± 2.41	11.22 ± 1.04	35.0 ± 6.0	210.0 ± 32.0	3.90	0.014*
Psorospernum febrifugum (C2)	12.42 ± 2.13	11.81 ± 1.23	36.0 ± 4.0	240.0 ± 25.0	4.12	0.018*
Ricinus communis (C3)	13.21 ± 2.50	11.03 ± 1.31	33.0 ± 5.0	250.0 ± 22.0	4.80	0.041*
Rosy periwinkle (C4)	14.01 ± 2.04	10.72 ± 1.13	32.0 ± 4.0	225.0 ± 30.0	3.95	0.020*
5-Fluorouracil Group (D)	15.02 ± 2.90	10.00 ± 1.03	34.0 ± 3.0	215.0 ± 25.0	4.60	0.015*

*Values are expressed as mean ± SD (n=6). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. *p < 0.05 compared with control/between groups as applicable.*

Table **2**. Effect of selected medicinal plant extracts on differential white blood cell count in benzene-induced haematopoietic dysfunction in Wistar rats

Group	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)	F-value	P value
Control (A)	60.0 ± 5.0	30.0 ± 4.0	5.0 ± 1.0	2.0 ± 0.5	1.0 ± 0.2	NS	N.S
Benzene-induced (B)	35.0 ± 6.0	50.0 ± 7.0	8 .0± 2.0	4.0 ± 1.0	3.0 ± 0.5	6.40	<0.001*
Azadirachta indica (C1)	52.0 ± 4.0	38.0 ± 5.0	6 ± 1	2.0 ± 0.3	2.0 ± 0.4	5.30	0.035*
Psorospernum febrifugum (C2)	50.0 ± 5.0	40.0 ± 6.0	6 ± 1	2.0 ± 0.5	2.0 ± 0.4	5.20	0.046*
Ricinus communis (C3)	48.0 ± 5.0	42.0 ± 6.0	6 ± 1	2.0 ± 0.3	2.0 ± 0.5	5.10	0.051*
Rosy periwinkle (C4)	49.0 ± 5.0	41.0 ± 5.0	5 ± 1	2.0 ± 0.4	3.0 ± 0.5	5.00	0.048*
5-Fluorouracil (D)	55.0 ± 4.0	35.0 ± 5.0	5 ± 1	3.0 ± 0.5	2.0 ± 0.3	5.50	0.024*

*Values are expressed as mean ± SD (n=6). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. *p <0.05.*

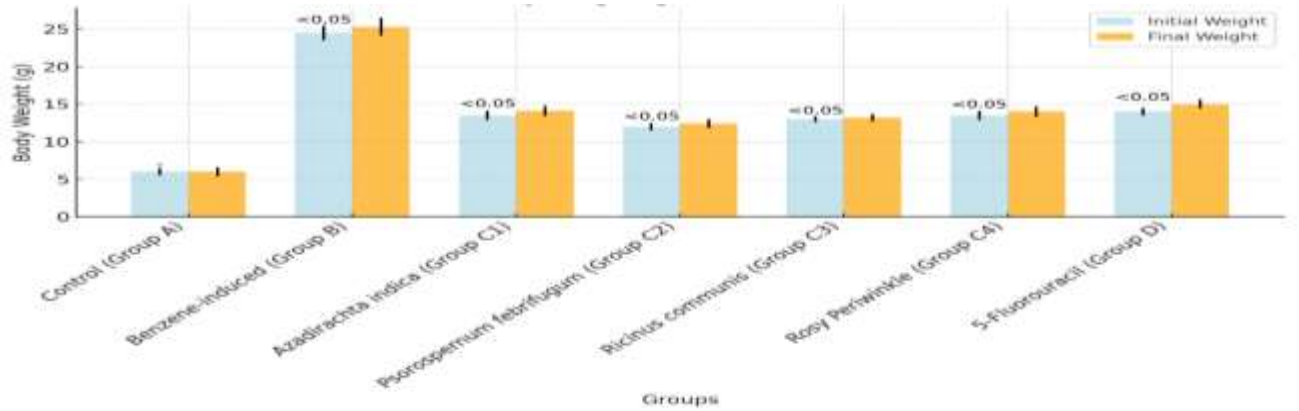

*Figure **1**. Changes in body weight of rats following benzene exposure and treatment with selected medicinal plant extracts.*

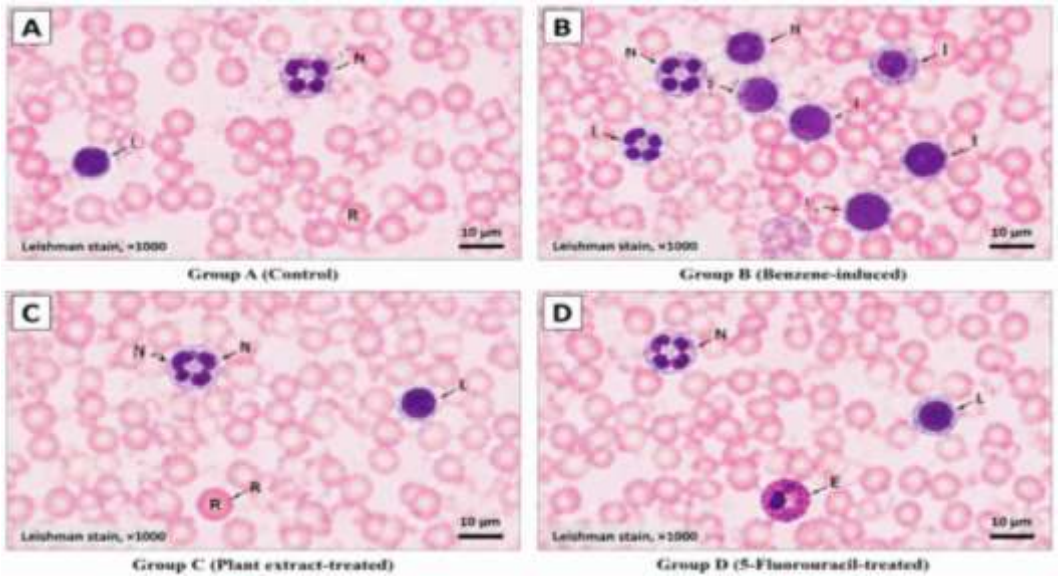

Figure 2. Representative peripheral blood film morphology of Wistar rats following benzene exposure and selected medicinal plant extract administration (Leishman stain, ×1000 magnification). Group A (Control): Arrows indicate normal erythrocytes, segmented neutrophils, and lymphocytes with preserved morphology. Group B (Benzene-induced haematopoietic dysfunction): Arrows indicate increased lymphocytes, atypical/immature lymphocyte-like cells, and altered leukocyte morphology. Group C (Plant extract-treated): Arrows indicate improved peripheral blood morphology with the presence of segmented neutrophils, lymphocytes, and normal erythrocytes. Group D (5-Fluorouracil-treated): Arrows indicate segmented neutrophils, lymphocytes, and eosinophils showing relatively preserved leukocyte morphology. R (Erythrocyte), N (Segmented neutrophil), L (Lymphocyte) I (Atypical/immature lymphocyte-like cell) and E (Eosinophil).

4.0 DISCUSSION

The body weight reduction triggered by benzene exposure may be an indicator of large scale systemic toxicity that is linked to benzene as it is being metabolised, or it may also represent some form of covariate with the general physiologic stress related to altered homeostasis. It has been reported that benzene can trigger several metabolic changes, oxidative stress and organ toxicity which may account for a lower growth rate and affect the body weight of experimental animals (Obazelu & Wasa, 2023; He et al., 2024). The partial recovery seen in the extract-treated groups may be due to various phytochemicals of medicinal origin, such as flavonoids and phenolic compounds, which are Antioxidants that may aid in chemical-induced cellular repair (Kooti et al., 2017). The protective effect of a herbal intervention against benzene toxicity has also been reported in experimental rats (Rehan et al., 2023).

Benzene-induced haematopoietic toxicity is mainly resulted from the generation of reactive metabolites that result from the bioactivation of benzene such as hydroquinone, catechol and benzoquinone. These metabolites generate oxidative stress, damage DNA, activate inflammatory signals and disturb regular haematopoietic stem and progenitor cells balance, thus influence the normal erythrocyte, leukocyte and platelet synthesis (Mc Hale et al., 2012; Chaachouay & Zidane, 2024). The changes observed in the haematological parameters of benzene exposed rats are consistent with the abnormalities associated with benzene exposure on haematopoietic activities (Cordiano et al., 2022; Obazelu & Omoregie, 2024). The modulatory effects could be associated to the phytochemical constituents, such as flavonoids, phenolic constituents, alkaloids and terpenoids, which demonstrated anti-inflammatory and antioxidant properties (Palhares et al., 2015; Mokwenye et al., 2023). These can contribute to diminishing oxidative damage by acting as free radical scavengers and effects that can inhibit the degradation of cellular components, as well as aiding the achievement of the recovery of haematopoietic dysfunction following chemical insult. The positive effects demonstrated by the extract-fed groups are thus indicative of mitigation of the benzene-induced haematological pathologies rather than reversal of a proven neoplastic process.

The differences in the response of plant extracts may be correlated with variations in their phytochemical profiles, bioactive constituent concentrations, and biological activities. *Azadirachta indica* has the bioactive constituents flavonoids and limonoids which are known for their antioxidant and immunomodulatory activity which may be responsible for protection against oxidative cellular injury in addition to other mechanisms (Kooti et al., 2017; Falini & Martelli, 2023). Various plant mediated protection against chemical induced physiological abnormalities have been described in Wistar rats, where *Acalypha wilkesiana* extract was also observed to prevent the degree of haematological abnormalities (Olubodun et al., 2024). The effects of *Ricinus communis* and *Psorospermum febrifugum* may also be related to the presence of known phenolic and other bioactive constituents in these plants, which may be due to their ability to act on oxidative and inflammatory pathways (Atere et al., 2026). The differences in magnitude of improvement across all the extract-treated groups may be due to variations in phytochemicals content, extract bioavailability and in vivo pharmacokinetic interactions with cellular components involved in haematopoietic recovery (Paulo et al., 2015). Extracts which showed the greatest correction with regards to erythrocyte and platelet parameters may have exerted a stronger cytoprotective activity, while extracts which exerted the greatest effect on leukocyte profiles may have exhibited a more potent immunomodulatory activity. Recently conducted experimental investigations support the notion that phytochemicals derived from medicinal plants can modify the haematological effects of benzene exposure (Heideveld & van den Akker, 2017; Barmoudeh et al., 2022; Obazelu & Wasa, 2024).

The peripheral blood film findings corroborated the haematological findings by showing morphological changes after benzene exposure, and improvements in cellular morphology after extract administration. These changes however should be interpreted with caution as peripheral blood film morphology alone is not diagnostic of leukaemia. Malignant disease can only be confirmed with examinations including bone marrow analysis, immunophenotyping or molecular characterisation.

Conclusion

This study shows that exposure to benzene induced marked changes in the haematological parameters and peripheral blood film, indicating that haematopoietic functions were grossly disrupted in the Wistar rats. The administration of the selected medicinal plants extracts (*Azadirachta indica* , *Psorospermum febrifugum*, *Ricinus communis*, and *Rosy periwinkle*) elicited various modulatory effects on the benzene related effects on haematology, thus can be linked to the antioxidant and anti-inflammatory effects of the phytochemical constituents found in the extracts. Based on the limitations of this work, the results suggest that the extracts investigated exhibited some degree of haematoprotective effects that can be used to counteract the effects of benzene exposure on haematopoietic functions. Further works should be pursued with phytochemical screening, mechanism studies and molecular assessments to identify the phyto-compounds and mechanisms involved.

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Competing Interests

Authors declare no conflicts of interest

Authors' contribution

KYA, AAD and AOI designed the study and drafted the first manuscript. KYA, AAD, ARO and AOI designed and reviewed the final manuscript for intellectual content. All authors contributed to the final version of the manuscript and are responsible for the integrity and accuracy of this review.

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