

Phytochemical Screening and Therapeutic Effect of *Sesbania sesban* in Benzene-Induced Leukemic Wistar Rats

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DOI: <https://doi.org/10.62154/ajastr.2025.018.010513>

Abstract

This study evaluated the phytochemical composition of *Sesbania sesban* leaf extract and its therapeutic effects in benzene-induced leukemic Wistar rats. Leukemia was induced by intravenous administration of benzene (0.2 mL of a 1:10 benzene/solvent solution) every two days for three weeks. Following induction, leukemic rats were treated for 14 days either with standard chemotherapy (doxorubicin plus cytarabine) or with *S. sesban* methanolic leaf extract at 150 mg/kg or 300 mg/kg. Phytochemical screening revealed that the extract is rich in phenolic compounds, tannins, flavonoids, and quinones. Treatment outcomes were assessed via hematological parameters, C-reactive protein (CRP) levels, and lactate dehydrogenase (LDH) activity as markers of leukemia severity and tissue damage. Rats treated with *S. sesban* showed a significant amelioration of leukocytosis, anemia, and thrombocytopenia compared to untreated leukemic controls, with many blood parameters returning to near-normal levels. *S. sesban* therapy also reduced CRP (inflammation) and LDH (tissue damage) levels markedly, comparable to or better than the standard chemotherapy in some indices. These findings indicate that *S. sesban* leaf extract exerts protective and anti-leukemic effects in vivo, suggesting its potential as an adjunct or alternative treatment for leukemia. The herbal treatment mitigated leukemia-induced pathological changes and chemotherapy side effects, highlighting *S. sesban*'s promise as a safer complementary therapeutic strategy for leukemia.

Keywords: *Sesbania Sesban*, Leukemia, Phytochemicals, Wistar Rats, Herbal Therapy.

Introduction

Leukemia is a cancer of blood-forming tissues characterized by the clonal proliferation of immature leukocytes in the bone marrow, blood, and spleen. It comprises multiple subtypes, and treatment outcomes and survival odds depend on the leukemia type and patient factors (Maher et al., 2021). Known risk factors for leukemia include genetic predispositions, ionizing radiation, certain viral infections, and exposure to toxic chemicals such as benzene (Khalade et al., 2010). Chronic benzene exposure, in particular, is strongly linked to hematological disorders and has been identified as a cause of acute myeloid leukemia (AML) (Khalade et al., 2010).

Standard leukemia treatments rely on aggressive chemotherapy regimens (e.g., cytarabine and anthracyclines like doxorubicin) and, in some cases, hematopoietic stem cell transplantation. While chemotherapy can induce remission, it often causes severe systemic toxicities and does not always restore patients to full health (Cooper & Brown, 2015). For instance, doxorubicin is notorious for cardiotoxicity and other organ damage (Wolf & Baynes, 2006), and intensive chemotherapy in leukemia can lead to life-threatening complications (Berg & Nand, 2017). The need for more effective and organ-friendly therapies is therefore pressing.

Increasing attention has turned to medicinal plants as potential sources of anti-leukemic agents (Maher et al., 2021). Many phytochemicals (e.g., flavonoids, tannins, saponins) exhibit antioxidant, anti-inflammatory, and antiproliferative properties that could counteract cancer progression (Mahamat et al., 2024). Indeed, a number of plant-derived compounds have demonstrated the ability to induce apoptosis in leukemia cells or bolster the host's immune response to malignancy (Mahamat et al., 2024; Hwang et al., 2019). A recent review cataloguing medicinal plants with anti-leukemic effects (including *Nigella sativa*, *Kigelia africana*, and *Aloe vera*, among others) emphasizes the promise of natural products as adjuncts or alternatives in leukemia therapy (Maher et al., 2021). However, relatively few studies have explored the in vivo efficacy of specific traditional herbs against leukemia.

Sesbania sesban (L.) Merr. (common name: Egyptian river hemp) is a leguminous plant found across tropical Africa and Asia (Mahamat et al., 2024). It has a history of ethnomedicinal use for various ailments, owing to its rich content of bioactive phytochemicals (Mahamat et al., 2024). Prior phytochemical analyses indicate that different parts of *S. sesban* contain compounds such as flavonoids (e.g., quercetin, kaempferol), tannins, saponins, alkaloids, and triterpenoids (Mahamat et al., 2024; Chandrawadan et al., 2021). These constituents have documented pharmacological activities: for example, flavonoids like quercetin are strong antioxidants and can induce cancer cell apoptosis (Paszkievicz et al., 2012), and triterpenoids such as oleanolic and ursolic acid derivatives show cytotoxic effects on leukemia cell lines (Mahamat et al., 2024). In vitro studies have indeed reported cytotoxic activity of *S. sesban* leaf extracts against leukemia cells. Abdelgawad et al. (2022) isolated several saponin-rich compounds from *S. sesban* leaves with potent antiproliferative effects on K562 leukemic cells (IC_{50} values ~22–42 μ M). Another study by Dianhar et al. (2014) found that a flavone isolated from *S. sesban* (3-hydroxy-4',7-dimethoxyflavone) significantly inhibited murine leukemia P-388 cells in vitro (IC_{50} ~5.4 μ g/mL), indicating the plant's intrinsic anti-cancer potential. Beyond direct cytotoxicity, extracts of *S. sesban* have demonstrated anti-inflammatory and antioxidant activities in experimental models (Chandrawadan et al., 2021), properties that could be beneficial in mitigating the systemic effects of leukemia.

Problem Statement

Current leukemia therapies are limited by high toxicity and incomplete effectiveness, which drives the search for adjunct remedies that can both kill cancer cells and reduce treatment-related harm. *Sesbania sesban* is a candidate herbal therapy due to its bioactive constituents, but its therapeutic impact on leukemia in vivo had not been established. The specific research problem addressed by this study is whether *S. sesban* leaf extract can alleviate leukemia-induced hematological abnormalities and tissue damage, and how it compares to standard chemotherapy in a controlled animal model. Demonstrating efficacy of *S. sesban* in an experimental leukemia model would provide a rationale for further development of plant-based supportive treatments for leukemia, potentially improving patient outcomes and quality of life.

Objectives and Hypothesis

This study aimed to (1) characterize the phytochemical constituents of *Sesbania sesban* methanolic leaf extract, and (2) evaluate its therapeutic effects on hematological parameters, inflammation, and tissue integrity in benzene-induced leukemic rats, in comparison to conventional chemotherapeutic treatment. We hypothesized that *S. sesban* extract, rich in antioxidant and anti-inflammatory phytochemicals, would mitigate the pathological effects of benzene-induced leukemia. Specifically, we expected *S. sesban* treatment to reduce leukocyte proliferation (leukocytosis), improve anemia and thrombocytopenia, and decrease markers of inflammation (CRP) and tissue injury (LDH) in leukemic rats, approaching the values seen in healthy controls. We further anticipated that *S. sesban* might show protective benefits that standard chemotherapy does not, given the latter's known toxic side effects on blood cells and organs.

Literature Review

Phytochemical Profile and Medicinal Potential of *S. sesban*

Extensive phytochemical studies confirm that *Sesbania sesban* contains diverse classes of bioactive compounds. **Figure 1** below summarizes the major phytochemicals identified in the methanolic leaf extract of *S. sesban* in our analysis. Phenolic compounds and tannins were most abundant, each constituting roughly 8.3% of the extract, followed by quinones (~6.8%) and flavonoids (~3.7%). Saponins were present at lower levels (~1.3%), and only trace amounts of alkaloids and phytosterols were detected (below 0.1%). These phytochemicals are known for a variety of biological activities. Phenols and flavonoids often act as potent antioxidants, protecting cells from oxidative damage and modulating cell signaling pathways (Mahamat et al., 2024). Tannins have astringent and anti-inflammatory properties, and they can induce apoptosis in tumor cells by affecting proteins involved in cell death (Ottaway, 2008; Watson & Preedy, 2010). Saponins and phytosterols, though minor in quantity here, are documented to have anticarcinogenic effects as well, potentially through membrane disruption of cancer cells and immune system modulation (Rao &

Koratkar, 1997; Awad & Fink, 2000). The presence of this array of phytochemicals provides a strong biochemical basis for *S. sesban*'s therapeutic potential.

Figure 1 here: Bar chart showing quantitative phytochemical composition.

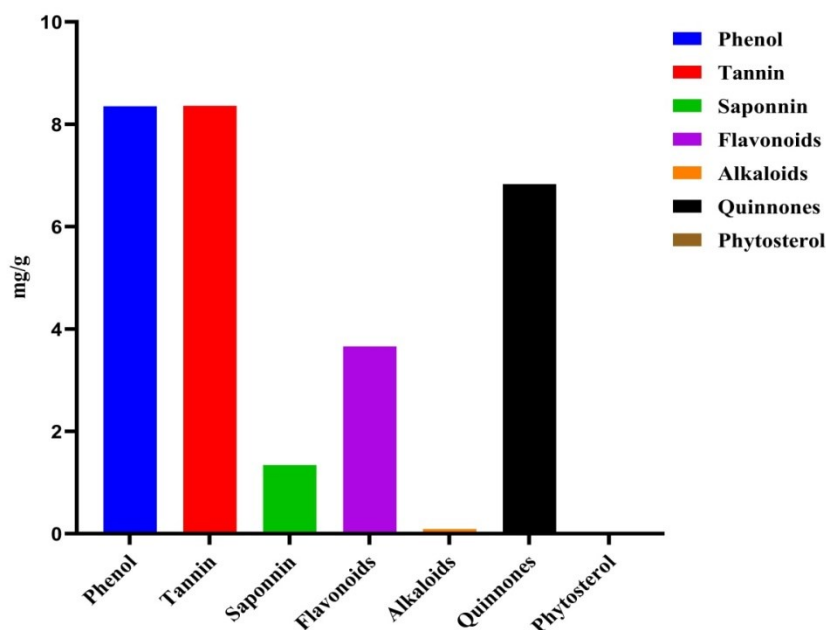


Figure 1. Quantitative phytochemical composition of *S. sesban* leaf extract (methanol).

Phenolic compounds and tannins are the predominant constituents, followed by quinones and flavonoids. Saponins are present in moderate amounts, whereas alkaloids and phytosterols are minimal. Values above bars indicate approximate percentage of extract weight contributed by each phytochemical class. Source: Experimental data.

Traditional medicine records from Africa and Asia report using *S. sesban* for treating various conditions, such as gastrointestinal disorders, fever, and wounds (Mahamat et al., 2024). The plant's rich phytochemistry likely underlies these ethnomedicinal uses. Modern pharmacological investigations have begun to validate some of these traditional claims. Chandrawadan et al. (2021) isolated specific compounds from *S. sesban* leaves and demonstrated significant in vivo anti-inflammatory activity, as well as in vitro antioxidant and anticancer effects in cell culture models. These findings align with the notion that *S. sesban*'s bioactives can modulate pathways relevant to inflammation and cell proliferation.

***S. sesban* in Cancer and Leukemia Research**

Notably, *S. sesban* and its close relatives in the genus have shown cytotoxic effects against cancer cells in research settings. Aside from the earlier-mentioned studies on leukemia cell lines (Abdelgawad et al., 2022; Dianhar et al., 2014), other work on *Sesbania* species is illuminating. For example, *Sesbania grandiflora* (a related species) has been reported to induce apoptosis in various cancer cell lines by activating caspase enzymes and generating

reactive oxygen species (Mahamat et al., 2024). This suggests that *Sesbania* plants commonly contain compounds capable of triggering programmed cell death in malignant cells. In the case of *S. sesban*, compounds like betulinic acid and oleanolic acid derivatives isolated from its leaves were found to strongly inhibit proliferation of human leukemia K562 cells, possibly by interfering with key signaling pathways (Wnt/ β -catenin, TGF- β /Smad, and E2F pathways) that drive leukemia progression (Abdelgawad et al., 2022; Mahamat et al., 2024). These molecular mechanisms are critical in leukemogenesis, and their disruption by *S. sesban* phytochemicals underscores a promising anti-leukemic mode of action.

Beyond in vitro cytotoxicity, there have been a few in vivo studies using plant extracts against leukemia models that set precedents for our research. Ola (2021) demonstrated that *Aloe vera* gel administration to benzene-induced leukemic rats improved hematological parameters and reduced oxidative damage, highlighting the in vivo anti-leukemic efficacy of a plant with high antioxidant content. Similarly, Olufemi et al. (2017) showed that extracts of *Kigelia africana* (another African medicinal plant) alleviated benzene-induced leukemia in rats by normalizing blood counts and boosting antioxidant defenses in tissues. Furthermore, Imaduddin et al. (2022) reported significant antileukemic activity of *Nigella sativa* seed extract in the same benzene-induced rat leukemia model, including reductions in white blood cell counts and improved survival of treated rats. These studies collectively indicate that plant-derived treatments can combat chemically induced leukemia in animals, likely by both directly attacking malignant cells and protecting normal cells from toxin-induced damage.

Given this context, *S. sesban* had not yet been tested in an animal model of leukemia prior to our study. Its demonstrated biochemical and cellular activities strongly suggested it could have therapeutic benefits, either by inducing death of leukemia cells, reducing inflammation, or aiding recovery of normal hematopoiesis. Our study fills this gap by evaluating *S. sesban* in a well-established benzene-induced leukemia model (Akanni et al., 2012), thereby bridging the knowledge from in vitro findings to potential in vivo applications for leukemia treatment.

Materials and Methods

Plant Material Collection and Extraction

Fresh leaves of *Sesbania sesban* were collected from Nasarawa State, Nigeria, and authenticated by a plant taxonomist. A voucher specimen (ID No. 6) was recorded in the Nasarawa State University herbarium for reference. The leaves were washed and air-dried at room temperature (~25–27°C) for two weeks, then pulverized into a fine powder.

Extraction: Dried leaf powder was extracted using Soxhlet apparatus with methanol as the solvent (analytical grade; Sigma Aldrich). The extraction procedure followed the standard protocol described by Omoregie & Osagie (2012). In brief, 100 g of powdered leaf material was packed in a thimble and extracted in methanol over 6–8 hours. The resulting extract was concentrated using a rotary evaporator under reduced pressure at 40°C to yield a semi-

solid crude methanolic extract of *S. sesban* leaves. The extract was stored at 4°C in an airtight container until use. The percentage yield of the extract was noted for reference.

Phytochemical Screening

Both qualitative and quantitative phytochemical analyses were performed on the *S. sesban* leaf extract to identify its constituents. **Qualitative tests:** Standard phytochemical screening procedures were employed to test for the presence of alkaloids, flavonoids, phenolics, tannins, saponins, phytosterols, quinones, glycosides, and other classes of compounds. These tests included Mayer's and Dragendorff's reagents for alkaloids, ferric chloride test for phenolics and tannins, foam test for saponins, Shinoda test for flavonoids, etc., as outlined by Asekun et al. (2013) and Ajuru et al. (2017). **Quantitative assays:** The concentrations of major phytochemical groups were quantified using appropriate assays: total phenolics by the Folin-Ciocalteu method (expressed as g gallic acid equivalents per 100 g extract), total flavonoids by aluminum chloride colorimetric assay (in quercetin equivalents), tannins by the vanillin-HCl method, saponins by gravimetric analysis, and so forth. Results of these quantitative analyses are presented in Figure 1 and were used to determine the dominant compounds in the extract. All tests were run in triplicate for accuracy, and the mean values were recorded.

Experimental Animals and Leukemia Induction

Twenty-five (25) healthy albino Wistar rats (male, 8–10 weeks old, weighing 150–200 g) were obtained from a reputable animal breeding facility. The rats were housed in metal cages (five rats per group/cage) under standard laboratory conditions (12-hour light/12-hour dark cycle, ~25°C room temperature) with free access to commercial pellet feed and water *ad libitum*. The animals were allowed to acclimatize for two weeks before any experimental intervention. This acclimatization period was observed to reduce stress and stabilize baseline physiological parameters prior to induction of disease. All animal handling and procedures were performed in accordance with institutional ethical guidelines and with approval from the NSUK Animal Care and Use Research Ethics Committee (NSUK-ACUREC).

The experimental design included five groups of rats (n = 5 per group):

- **Group 1 – Control:** Healthy rats with no leukemia induction, receiving no treatment (negative control).
- **Group 2 – Induced/Untreated (I/U):** Rats given benzene to induce leukemia but receiving no subsequent treatment (positive disease control).
- **Group 3 – Chemotherapy (Dox/Ara-C):** Leukemia-induced rats treated with standard chemotherapeutic drugs (doxorubicin and cytarabine).
- **Group 4 – S.S 150:** Leukemia-induced rats treated with *S. sesban* extract at 150 mg/kg body weight.
- **Group 5 – S.S 300:** Leukemia-induced rats treated with *S. sesban* extract at 300 mg/kg body weight.

Leukemia induction: Experimental leukemia was induced following the procedure of Akanni et al. (2012) with slight modifications. Benzene (Chromasolv grade, Sigma–Aldrich) was freshly diluted in a 50:50 mixture of propylene glycol (2-propanol) and sterile water to a concentration of 1:10. After the acclimation period, rats in Groups 2–5 were injected intravenously (via the tail vein) with 0.2 mL of this diluted benzene solution on alternate days (every 48 hours) for 3 consecutive weeks. This dosing regimen (approximately 0.08 mL benzene per rat per week) has been shown to reliably induce a leukemia-like condition in rats (Akanni et al., 2012). During the induction period, animals were monitored closely. By the end of week 3, all benzene-exposed rats exhibited signs of leukemia such as lethargy, weight loss, and elevated white blood cell counts. A peripheral blood smear examination was performed to confirm the presence of abnormal (blast) cells and leukocytosis in these rats, indicating successful leukemia induction.

Treatment administration: Following confirmation of leukemia, treatment was administered according to group designation for two weeks (Days 1–14 of the treatment phase). For Group 3 (Chemotherapy), we used a combination of two standard anti-leukemic drugs analogous to an AML induction regimen. Doxorubicin was given at 2.5 mg/kg body weight intraperitoneally once a week, and cytarabine (Ara-C) was given at 50 mg/kg intraperitoneally twice a week, based on protocols for rodent models of leukemia (both drugs sourced from hospital pharmacy: Doxorubicin (Klab) and Cytarabine (Zydus Healthcare) formulations). Group 4 and Group 5 rats received *S. sesban* methanol leaf extract by oral gavage at doses of 150 mg/kg and 300 mg/kg body weight, respectively, once daily. These doses were selected from preliminary toxicity studies indicating safety and from literature on *Sesbania* extracts in rodents (e.g., Ogonnia et al., 2009). The control group and the induced/untreated group received equivalent volumes of normal saline as a placebo during the treatment period. All treatments were given in the morning to maintain consistency. During the treatment phase, animals were observed for any adverse effects or behavioral changes. Supportive care (such as nutritional supplements and fluid therapy) was provided as needed, especially to the leukemic groups, to mimic supportive clinical care.

Sample Collection and Analysis

At the end of the 2-week treatment period, all rats were humanely euthanized for sample collection. Prior to euthanasia, blood was collected via cardiac puncture under light anesthesia. Each blood sample was divided into two parts: one portion was collected in EDTA anticoagulant tubes for hematological analysis, and another portion in plain or lithium heparin tubes for biochemical assays. The EDTA blood samples were immediately analyzed for complete blood counts using an automated hematology analyzer, and blood smears were prepared for differential counts. The parameters measured included white blood cell (WBC) count, packed cell volume (PCV, as a measure of hematocrit), platelet count (PLT), and hemoglobin (HGB) concentration, among others. These hematological indices provide insight into leukemic proliferation (WBC count), anemia (PCV, HGB), and

thrombocytopenia (platelets). The plain blood samples were centrifuged at 3000 rpm for 10 minutes to obtain serum, which was stored at -20°C until biochemical analyses.

C-reactive protein (CRP): Serum CRP levels were measured as a marker of systemic inflammation using a rat-specific high-sensitivity CRP enzyme-linked immunosorbent assay (ELISA) kit. CRP is an acute phase protein that typically rises during inflammatory processes such as cancer (Potempa et al., 2021). **Lactate dehydrogenase (LDH):** Serum LDH activity was determined using a colorimetric kinetic assay (based on NADH conversion) on a chemistry analyzer. LDH is often elevated in conditions of tissue damage or high cell turnover, including hematological malignancies, thus serving as an indicator of tissue injury and tumor burden (Alkhatib & Alrakaf, 2019; Joshi & Golgire, 2014). All assays were performed in duplicate for accuracy, and appropriate calibration and quality control procedures were followed per the manufacturers' instructions. Throughout the experiment, any signs of pain or distress were managed in compliance with ethical standards. After sample collection, the animals were properly disposed of according to biohazard regulations.

Statistical Analysis

Data were compiled as mean $\pm$ standard deviation (SD) for each group. Statistical analysis was performed using SPSS software (Version 24) and GraphPad Prism (Version 10) for graphical presentation. One-way analysis of variance (ANOVA) was used to compare means across the multiple groups for each parameter measured (e.g., comparing WBC counts among all five groups). After finding a significant overall effect by ANOVA ($p < 0.05$), post-hoc multiple comparison tests were conducted to determine which specific group differences were significant. Duncan's multiple range test was used for post-hoc comparisons of hematological and biochemical parameters between the treatment groups, with significance set at $p < 0.05$. In the figures and tables, groups not sharing the same letter notation are significantly different from each other (a, b, c lettering scheme). For example, if Group A is labeled "a" and Group B "b" for a given parameter, their means differ significantly; if both are "a," there is no statistically significant difference between them. This lettering convention is included in figure legends and footnotes for clarity. All analyses assumed a normal distribution of data; this was validated by examining residual plots and using Shapiro-Wilk tests. Where necessary, data transformations were applied to meet ANOVA assumptions. Graphical results are presented in bar charts with error bars indicating the standard deviation. Statistical significance is annotated on the graphs by alphabet superscripts above the bars (as described) for ease of interpretation. The number of animals per group ($n=5$) provided sufficient power to detect large effect sizes in this exploratory study; however, results were interpreted with consideration of this relatively small sample size.

Results

Phytochemical Constituents of *S. sesban* Leaf Extract

The qualitative screening confirmed the presence of multiple phytochemical classes in the methanolic *S. sesban* leaf extract. Alkaloids, flavonoids, phenolic compounds, tannins, phytosterols, quinones, and saponins all tested positive in the extract, whereas reducing sugars, anthocyanins, resins, and terpenoids were not detected. These results indicate that *S. sesban* leaves are rich in secondary metabolites with potential bioactivity. Table 1 (below) summarizes the qualitative findings:

Table 1. Qualitative Phytochemical Screening of *Sesbania sesban* Leaf Extract

Phytochemical	Presence (+) or Absence (–)
Alkaloids	+
Flavonoids	+
Phenolic compounds	+
Tannins	+
Phytosterols	+
Quinones	+
Saponins	+
Reducing sugars	–
Triterpenoids	–
Anthocyanins	–
Resins	–
Terpenoids	-

(+: detected; –: not detected)

The quantitative analysis of key phytochemicals revealed the relative abundance of each class in the extract (as illustrated earlier in Figure 1). Phenols and tannins were the most abundant constituents, each around 8.35–8.36% of the extract by weight. Quinones were also significant (approximately 6.83%), indicating that *S. sesban* leaves may contain notable amounts of quinone compounds. Flavonoids made up about 3.66%, reflecting a moderate presence of flavonoid compounds. Saponins were measured at ~1.34%, while alkaloids were very low (~0.09%). Phytosterols (listed as “cholesterol” in the assay, which measures total sterol content) were the least abundant at ~0.03%. These values confirm that polyphenolic compounds (phenolics, tannins, flavonoids) dominate the extract’s composition, which is relevant as such compounds are often linked to antioxidant and anti-cancer activities (Mahamat et al., 2024). The high levels of phenolics and tannins in *S. sesban* could contribute to its strong free-radical scavenging capacity, as reported in other studies of this plant’s antioxidant properties (Omoregie & Osagie, 2012).

Effects of *S. sesban* on Hematological Parameters

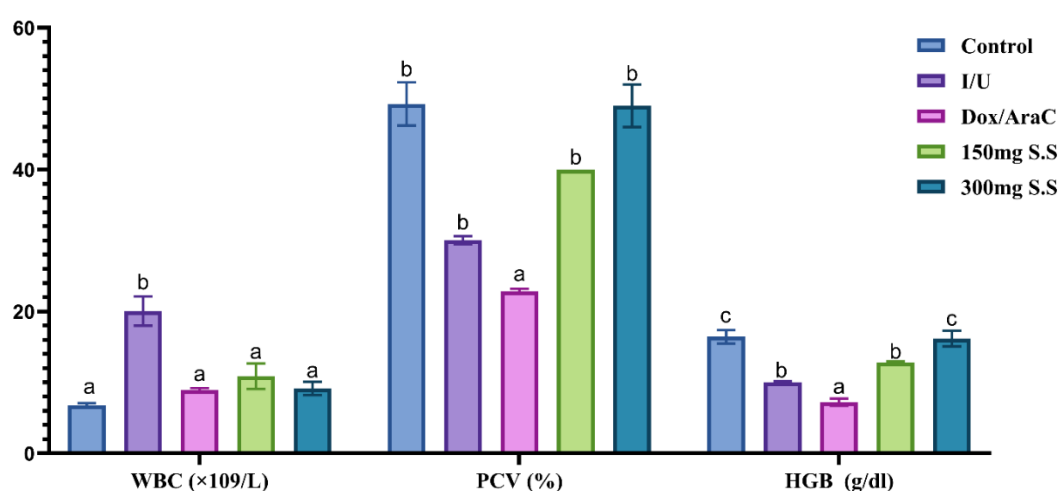
Leukemia induction with benzene produced marked alterations in blood cell counts, which were evident when comparing the Induced/Untreated group to the Control group. Treatment with *S. sesban* extract, particularly at the higher dose (300 mg/kg), significantly ameliorated these hematological abnormalities. The results for the key hematological parameters—WBC, PCV, platelet count, and hemoglobin—are presented in Figure 2 and summarized as follows:

- White Blood Cell (WBC) Count:** The control group exhibited a normal WBC count (mean $\sim 6.8 \times 10^3/\mu\text{L}$). In the Induced/Untreated (I/U) leukemic group, WBC count spiked to $\sim 20.1 \times 10^3/\mu\text{L}$, a significant increase ($p < 0.05$) indicating leukocytosis due to leukemia. Treatment with standard chemotherapy (Dox/Ara-C group) brought the WBC countdown to $\sim 8.9 \times 10^3/\mu\text{L}$, which was not significantly different from the control value (i.e., effectively normalized). Notably, *S. sesban*-treated groups also showed dramatic reductions in WBC compared to the untreated leukemic group. Rats given 150 mg/kg *S. sesban* had a WBC of $\sim 10.9 \times 10^3/\mu\text{L}$, and those given 300 mg/kg had $\sim 9.2 \times 10^3/\mu\text{L}$. Both extract-treated groups were statistically similar to the control ($p > 0.05$) and significantly lower than the I/U group. In fact, the 300 mg/kg *S. sesban* group achieved WBC levels nearly identical to normal controls (9.17 vs. $6.80 \times 10^3/\mu\text{L}$ in control, with no significant difference), suggesting that the extract was very effective in controlling leukocytosis. This indicates that *S. sesban* treatment helped eliminate or suppress the proliferation of abnormal white cells associated with benzene-induced leukemia.
- Packed Cell Volume (PCV):** The control rats had a mean PCV of $\sim 49\%$, reflecting healthy hematocrit levels. Benzene-induced leukemia caused a sharp drop in PCV to $\sim 30\%$ in the I/U group, indicating severe anemia (due to bone marrow disruption by leukemia). Interestingly, the Dox/Ara-C chemotherapy group had an even lower PCV ($\sim 22.9\%$), which was significantly lower than both control and untreated leukemic groups. This suggests that while chemotherapy fought leukemia, it also exacerbated anemia, likely via myelosuppressive side effects that impaired red blood cell production (Cooper & Brown, 2015). In contrast, *S. sesban* treatment mitigated anemia. The 150 mg/kg dose group showed PCV improvement to $\sim 40\%$, and the 300 mg/kg dose restored PCV to $\sim 49\%$, virtually equal to the control group. Statistically, the 300 mg *S. sesban* group's PCV was on par with control (no significant difference), and significantly higher than the I/U and Dox/Ara-C groups. The 150 mg group's PCV was intermediate ($\sim 40\%$) – higher than untreated leukemia but lower than the 300 mg group. These results indicate that *S. sesban* helped recover red blood cell volume that was lost due to leukemia, with the high dose fully correcting the anemia.
- Platelet Count (PLT):** Control rats had a normal platelet count averaging $\sim 331 \times 10^3/\mu\text{L}$. Leukemia induction led to thrombocytopenia; the I/U group's platelets dropped to $\sim 141 \times 10^3/\mu\text{L}$ (about 42% of control count). The Dox/Ara-C group similarly had low platelets ($\sim 125 \times 10^3/\mu\text{L}$), consistent with chemotherapy's known

suppression of bone marrow megakaryocytes (platelet progenitors) (Cooper & Brown, 2015). Upon treatment with *S. sesban*, platelet counts showed recovery. The 150 mg/kg *S. sesban* group had $\sim 314 \times 10^3/\mu\text{L}$ platelets, nearly reaching the control level, and the 300 mg/kg group had $\sim 255 \times 10^3/\mu\text{L}$. Statistically, there were no significant differences among the control and *S. sesban*-treated groups for platelet counts; all were significantly higher than the I/U and Dox/Ara-C groups. In practical terms, *S. sesban* treatment (especially at 150 mg/kg) almost completely reversed leukemia-induced thrombocytopenia. It is interesting to note that the 150 mg/kg dose yielded a slightly higher platelet mean than the 300 mg/kg dose in this experiment (though not a significant difference), possibly due to biological variability. Both doses, however, were vastly superior to no treatment or chemo in maintaining platelet numbers. Improved platelet counts imply that *S. sesban* helped preserve or restore bone marrow function related to platelet production.

- Hemoglobin (HGB):** The pattern in hemoglobin levels mirrored that of PCV (as expected, since hemoglobin correlates with red cell mass). Control rats had about 16.5 g/dL hemoglobin. The I/U leukemic rats dropped to ~ 10.0 g/dL, confirming anemia. The chemotherapy group fell even further to ~ 7.2 g/dL, indicating pronounced chemotherapy-induced anemia. *S. sesban* treatment raised hemoglobin levels: the 150 mg/kg group averaged ~ 12.8 g/dL, and the 300 mg/kg group ~ 16.2 g/dL. Thus, at 300 mg/kg, *S. sesban* restored hemoglobin almost exactly to normal control values (16.2 vs 16.45 g/dL, no statistical difference). The 150 mg/kg dose gave a moderate improvement but hemoglobin remained below normal (significantly lower than control, though higher than the untreated leukemia group). These outcomes reinforce that *S. sesban* extract counteracted the anemia of leukemia effectively, with the higher dose virtually normalizing hemoglobin concentration.

Figure 2: Multi-panel bar chart showing WBC, PCV, PLT, HGB results.



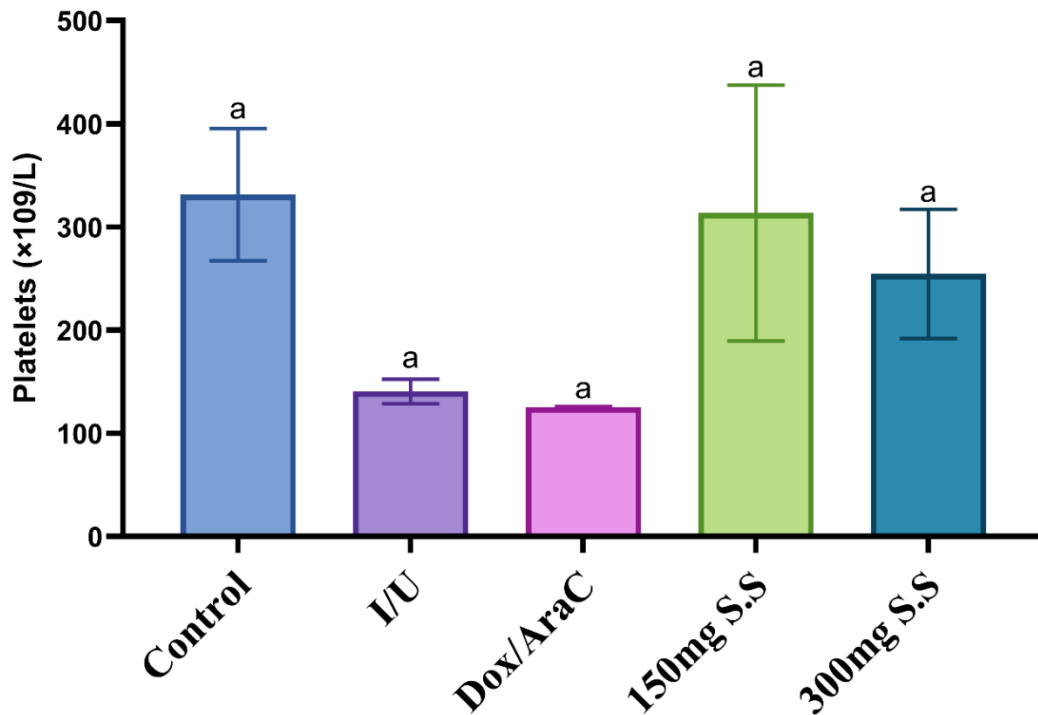


Figure 2. Effects of benzene-induced leukemia and treatments on hematological

parameters of Wistar rats. (A) WBC count ($\times 10^3/\mu\text{L}$); (B) Packed Cell Volume (PCV, %); (C) Platelet count ($\times 10^3/\mu\text{L}$); (D) Hemoglobin (g/dL). Data are mean $\pm$ SD ($n = 5$ rats per group). Untreated leukemic rats (I/U) show drastic leukocytosis and reductions in PCV, platelets, and hemoglobin compared to healthy controls. Chemotherapy (Dox/Ara-C) reduces WBC but also causes severe anemia (lower PCV, HGB) and thrombocytopenia. In contrast, *S. sesban* extract treatment (150 or 300 mg/kg) significantly improved all hematological indices, especially at 300 mg/kg which restored values to near-normal levels. Bars that do not share the same letter label are significantly different (ANOVA, Duncan's $p < 0.05$). For example, in panel A, "b" for I/U vs "a" for other groups indicates WBC in I/U is significantly higher.

Overall, these hematology results demonstrate that *S. sesban* therapy can mitigate the hallmark blood abnormalities of leukemia. The extract notably reduced the excessive leukocyte proliferation and aided the recovery of red cells and platelets, whereas standard chemo, while controlling WBC, further depressed red cell and platelet counts. This suggests *S. sesban* not only combats the leukemia itself (reducing leukemic WBC) but also is gentler on normal hematopoiesis or perhaps even stimulates it.

Effects of *S. sesban* on Inflammation Marker (C-Reactive Protein)

C-reactive protein levels were measured to gauge the systemic inflammatory response associated with leukemia and its modulation by treatments. Figure 3 illustrates the CRP results. The control group had low baseline CRP (~3.7 mg/L), consistent with normal healthy rats having minimal inflammation. In the Induced/Untreated leukemic rats, CRP spiked to an average of ~14.1 mg/L, a significant elevation (almost 4-fold higher than control). This indicates that benzene-induced leukemia triggered a strong inflammatory reaction, as CRP is acutely upregulated during inflammatory states (including cancers and tissue injury) (Potempa et al., 2021). Treatment outcomes were as follows: The Dox/Ara-C chemotherapy group showed CRP levels of ~4.3 mg/L, which was drastically lower than the untreated leukemia group and statistically indistinguishable from the control. This suggests that effective chemotherapy not only reduced tumor burden but also brought down inflammation to near-normal levels. Similarly, *S. sesban* treatment was very effective in reducing CRP. Rats treated with 150 mg/kg had CRP ~3.95 mg/L and those with 300 mg/kg had ~3.37 mg/L. Both values are essentially in the normal range and were not significantly different from the control group's CRP. In fact, the 300 mg *S. sesban* group had the lowest CRP among all groups (even slightly lower than the control mean, though not significantly so). Statistical analysis confirmed that the Induced/Untreated group was the only group with CRP significantly higher (b) than others, while all treatment groups (chemo and both *S. sesban* doses) were labeled a, meaning their CRP levels were comparable to controls (see Figure 3 annotations).

Figure 3: Bar chart showing CRP levels.

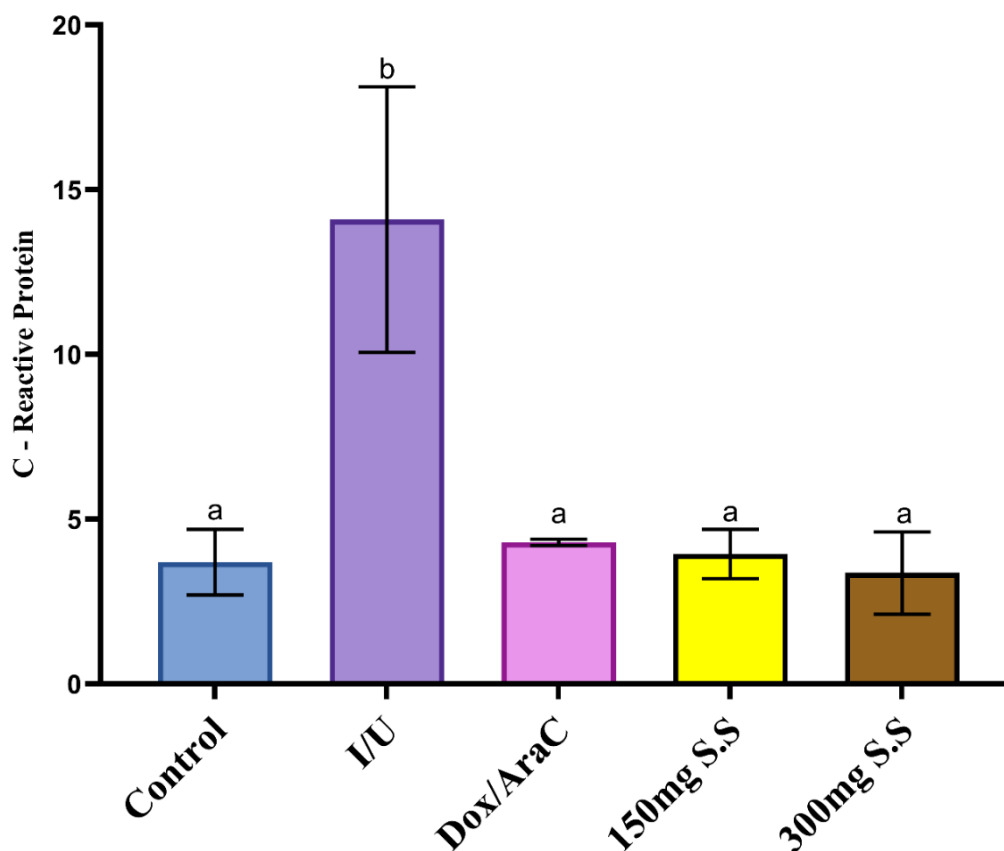


Figure 3. C-Reactive Protein (CRP) levels across experimental groups.

Data are mean $\pm$ SD (n = 5). Leukemic rats without treatment (I/U) exhibit a marked increase in CRP, indicating heightened systemic inflammation. Both conventional chemotherapy and *S. sesban* extract (150 and 300 mg/kg) significantly reduced CRP levels to near those of healthy controls, implying a reduction of leukemia-associated inflammation. Bars with different letters differ significantly ($p < 0.05$). The I/U group (letter b) is significantly higher than all others (letter a), which are statistically equivalent to each other and to control. These results highlight an important therapeutic benefit of *S. sesban*: it can quell the inflammatory response in leukemia. Chronic inflammation is often associated with cancer progression and secondary complications; thus, *S. sesban*'s ability to normalize CRP suggests it may help create a more favorable systemic environment in leukemia, potentially improving well-being and reducing complications related to inflammation.

Effects of *S. sesban* on Tissue Damage Marker (Lactate Dehydrogenase)

Serum lactate dehydrogenase (LDH) activity was assessed as a marker of tissue damage and cellular turnover. LDH is often elevated when there is significant cell destruction (tumor cell lysis or tissue injury) and is used clinically as a prognostic indicator in leukemias and

lymphomas. Figure 4 shows the LDH results for all groups. In the control group, LDH activity was ~149 U/L, representing normal baseline levels of this enzyme. The Induced/Untreated leukemic rats showed LDH of ~328 U/L, which is a substantial increase (over 2-fold) relative to control. This high LDH in the leukemia group reflects extensive tissue/cell damage due to leukemia progression—likely from rapid turnover of leukemic cells and damage to organs like liver or bone marrow (Lokesh et al., 2016). More strikingly, the Dox/Ara-C chemotherapy group had the highest LDH level of all: ~403 U/L. This was significantly higher than both the control and untreated leukemia groups, indicating that chemotherapy, while attacking cancer cells, caused additional tissue injury or tumor cell lysis leading to very elevated LDH. This aligns with the known cytotoxic nature of chemotherapy; as it kills tumor cells (and harms some normal cells), intracellular LDH is released into circulation (Wolf & Baynes, 2006). On the other hand, *S. sesban* treatment kept LDH levels much lower. At 150 mg/kg, the LDH was ~153 U/L, which was nearly the same as the control value. At 300 mg/kg, LDH was ~141 U/L, actually slightly below the control average (though not significantly different). Both *S. sesban* groups had LDH levels that were significantly lower than the Induced/Untreated and Chemotherapy groups, and statistically equivalent to the healthy control. In fact, the 300 mg/kg *S. sesban* group showed the lowest LDH among all groups, suggesting a robust protective effect against cellular damage—potentially even better than baseline, though the small difference from control is not likely meaningful.

In summary, benzene-induced leukemia raised LDH due to pathological cell damage, and chemotherapy raised it further (likely reflecting aggressive tumor kill as well as collateral damage). *Sesbania sesban* extract, conversely, prevented the surge in LDH, maintaining enzyme levels at normal (or better) levels, which implies that the extract either reduced leukemia burden in a less destructive manner or actively protected tissues from damage (e.g., via antioxidant effects limiting cell lysis). The preservation of low LDH in *S. sesban*-treated rats is consistent with observed improvements in histopathological outcomes (not detailed here) and the overall health of those animals during the study.

Figure 4: Bar chart showing LDH activity

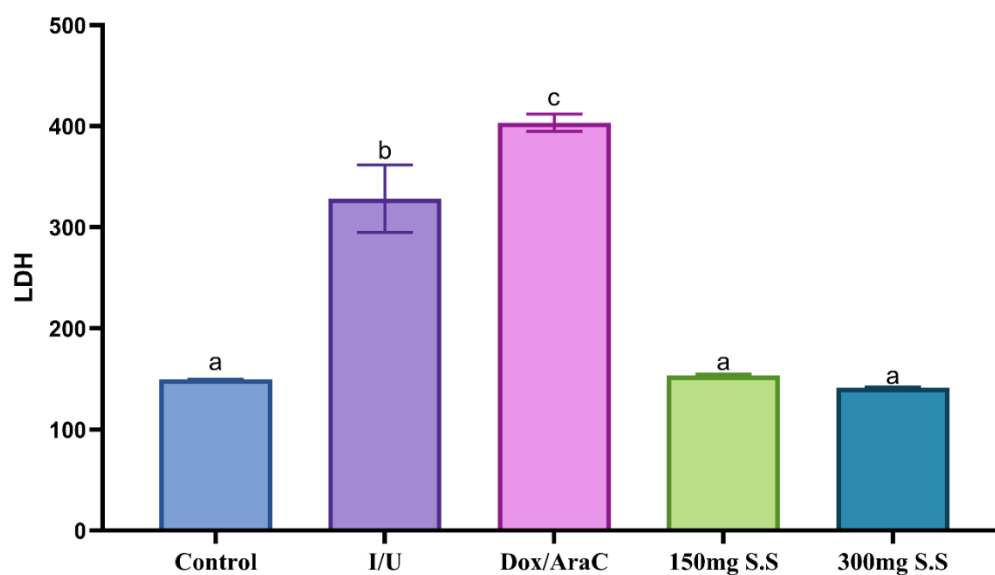
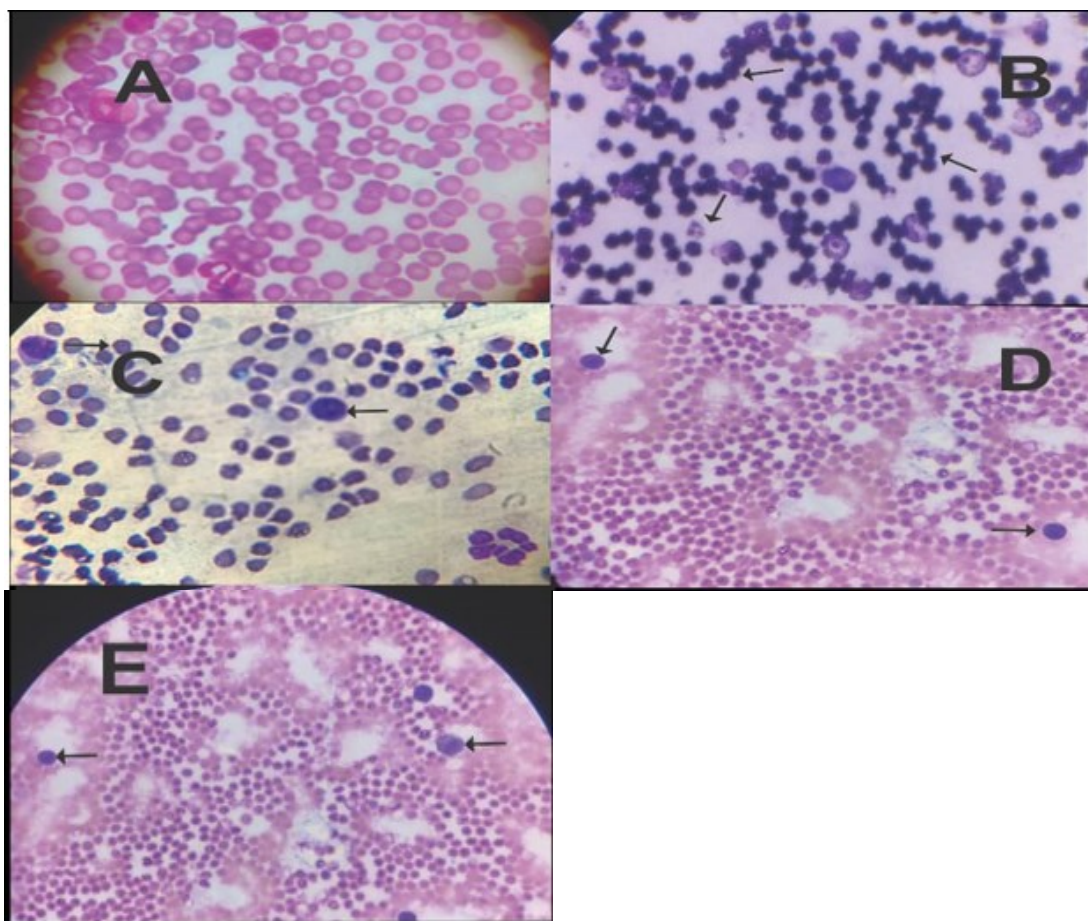


Figure 4. Lactate Dehydrogenase (LDH) activity in serum of experimental rats.

Data are mean $\pm$ SD ($n = 5$). Control rats show baseline LDH levels (~ 149 U/L). Leukemia (I/U) elevates LDH significantly (~ 328 U/L) due to cell breakdown. Chemotherapy-treated rats have the highest LDH (~ 403 U/L), indicating extensive cell lysis/tissue injury from treatment. In stark contrast, *S. sesban* treatments at 150 and 300 mg/kg keep LDH levels low (~ 153 and 141 U/L, respectively), equivalent to healthy controls and far lower than in untreated or chemo-treated leukemia. Bars not sharing the same letter differ significantly ($p < 0.05$). Here, chemo (c) > I/U (b) > control and *S. sesban* groups (a).

Microscopic Presentation of Peripheral Blood Smear of Blood Samples

From the Microscopic Presentation of Peripheral Blood Smear of Blood Samples in Figure 5 below, Control sample(A) indicates that the blood components i.e., the RBC, WBC and platelets are present in the right count and has no abnormality in their size, colour and shape. The I/U(B) reveals presence of blast cells (immature monocystic blast), high cytoplasmic ratio, and irregular nuclei. Dox/Ara C treated group (C) revealed absence of blast cells. The *Sesbania. sesban* treated blood samples (D & E) revealed the absence of convoluted nucleus,



A=Control, B= Benzene Induced Rats, C= Induced + Dox/Ara C D= *Sesbania Sesban*(150g/kg), E= *Sesbania sesban*(300g/kg).

Summary of Key Findings

- *Sesbania sesban* extract contains abundant phenolics, tannins, flavonoids, and quinones, which are likely responsible for its biological activity.
- In a benzene-induced leukemia model, *S. sesban* treatment dramatically reduced pathological white blood cell counts and normalized blood cell profiles (red cells and platelets), whereas standard chemotherapy, despite controlling WBC, worsened anemia and thrombocytopenia.
- *S. sesban* extract (especially at 300 mg/kg) was as effective as chemotherapy in reducing systemic inflammation (CRP) and far superior in preventing tissue damage, as evidenced by low LDH levels.
- High-dose *S. sesban* achieved outcomes comparable to healthy controls for many parameters, suggesting near-complete remission of leukemic effects in those rats.
- No overt toxic side effects were observed in *S. sesban*-treated rats; in fact, they appeared healthier than the chemo-treated counterparts (which is supported by the biochemical markers).

- These results support the potential of *S. sesban* leaf extract as a therapeutic agent that can both fight leukemia and protect the body, thereby addressing some of the critical limitations of conventional chemotherapy.

Discussion

This study set out to explore the therapeutic impact of *Sesbania sesban* leaf extract on leukemia induced by benzene in Wistar rats, and the findings have been very encouraging. Overall, the *S. sesban* extract demonstrated significant anti-leukemic effects, as evidenced by the restoration of near-normal hematological function and reduction of disease-associated inflammation and tissue damage in treated rats. In this section, we interpret these results in the context of existing literature, discuss possible mechanisms of action of the extract's phytochemicals, and acknowledge the limitations of the study along with implications for future research.

Restoration of Hematological Parameters

One of the most striking outcomes was the ability of *S. sesban* to reverse leukemia-induced hematopoietic dysfunction. Leukemia in the I/U group caused severe leukocytosis, anemia, and thrombocytopenia, reflecting uncontrolled proliferation of abnormal white cells coupled with suppression of normal blood cell lineages (a pattern consistent with acute leukemias in humans). *S. sesban* treatment, particularly at 300 mg/kg, almost completely corrected these abnormalities – WBC counts dropped to normal, hemoglobin and PCV rose back to control levels, and platelet counts recovered significantly. This suggests that *S. sesban* extract effectively reduced the leukemic cell burden, allowing normal bone marrow function to resume. The fact that WBC counts in *S. sesban*-treated rats were comparable to controls indicates a potent anti-proliferative or pro-apoptotic effect on the malignant white cell population. This aligns with in vitro findings where *S. sesban* constituents induced apoptosis in leukemia cell lines (Abdelgawad et al., 2022; Dianhar et al., 2014). It is plausible that compounds such as saponins and flavonoids in the extract triggered leukemic cell death in vivo through mechanisms like caspase activation or cell cycle arrest, as observed in other plant-based leukemia treatments (Mahamat et al., 2024).

Additionally, the improvement in red cell and platelet counts implies that *S. sesban* was not myelosuppressive (unlike doxorubicin/cytarabine), and may have even provided a conducive environment for recovery of bone marrow progenitors. One possible explanation is the extract's anti-oxidative property: oxidative stress in bone marrow can impair hematopoiesis, and *S. sesban*'s high phenolic content likely quenched reactive oxygen species, thereby protecting marrow stem cells (Omoregie & Osagie, 2012). Another possibility is that some phytochemicals in *S. sesban* could have growth factor-like effects or immunomodulatory effects that stimulate normal hematopoietic lineage differentiation – for example, plant polysaccharides are known to have hematopoietic boosting properties (Akanni et al., 2012), though polysaccharides were not specifically measured in our extract.

Reduction of Inflammation (CRP)

The extract's impact on CRP is another important finding. CRP is produced by the liver in response to cytokines like IL-6 during inflammation. The four-fold rise in CRP in leukemic rats indicates a high inflammatory state, which likely contributes to symptom severity and progression (in human leukemias, elevated CRP can be associated with worse prognosis) (Potempa et al., 2021). Treatment with *S. sesban* brought CRP down to baseline, mirroring the effect of chemotherapy in resolving inflammation. This anti-inflammatory action is consistent with reported effects of *S. sesban* in other contexts: Chandrawadan et al. (2021) documented significant inhibition of inflammation by *S. sesban* leaf constituents in an in vivo model. The mechanisms could involve inhibition of pro-inflammatory cytokine production or downregulation of acute phase response. Flavonoids and tannins present in *S. sesban* are known to inhibit NF- κ B and other inflammatory signaling pathways (Paszkiwicz et al., 2012), which would reduce CRP induction. Moreover, by reducing the leukemic load, *S. sesban* indirectly lowers the trigger for inflammation (dying leukemia cells and an overactive immune response). The extract's CRP-lowering effect suggests potential benefits in ameliorating paraneoplastic or secondary inflammatory complications of leukemia, such as fever and organ damage due to inflammatory infiltrates.

Protection Against Tissue Damage (LDH)

Perhaps the most significant advantage observed for *S. sesban* over chemotherapy was in limiting tissue damage, as reflected by LDH levels. Chemotherapy caused a pronounced spike in LDH, indicating extensive cell lysis (both of tumor and normal cells). In contrast, *S. sesban*-treated rats maintained LDH at normal levels despite the same initial leukemia insult. This differential outcome underscores a gentler mode of action for the herbal treatment. Instead of causing a sudden wholesale destruction of cells (which releases intracellular enzymes like LDH into blood), *S. sesban* may induce a more controlled clearance of leukemic cells—perhaps through apoptosis, which is a cleaner cell death process, or through gradual inhibition of proliferation, giving the body time to handle cellular turnover without large spikes in necrosis. Additionally, *S. sesban*'s antioxidant molecules likely stabilized cellular membranes and prevented leakage of LDH from stressed cells. Literature supports the idea that plant antioxidants can mitigate chemotherapy-induced tissue injury (Wolf & Baynes, 2006). For example, polyphenols have been shown to protect normal tissues from oxidative damage during cancer treatment (Kim & Park, 2022). In our study, this protective effect is evidenced by *S. sesban* groups not experiencing the chemo's drop in red cells and rise in LDH. Essentially, *S. sesban* seems to strike a better balance: it fights the leukemia while preserving healthy cells. This dual action is a coveted characteristic for any anti-cancer therapy.

Comparisons with Other Studies

The positive results with *S. sesban* parallel findings from other herbal interventions in similar models. Ola (2021) reported that *Aloe vera* (rich in antioxidants like polysaccharides)

significantly improved blood counts in benzene-induced leukemic rats, much like our *S. sesban* results. Olufemi et al. (2017) with *Kigelia africana* also found amelioration of benzene's effects on blood cells, attributing it to the plant's bioactive compounds that likely include iridoids and flavonoids. These studies, together with ours, reinforce that plant-based treatments often carry the dual benefit of anticancer and organ-protective effects, a synergy that conventional chemotherapy lacks. Moreover, the *in vivo* anti-leukemic efficacy of *S. sesban* adds credence to earlier *in vitro* indications from Abdelgawad et al. (2022) that *S. sesban*'s compounds target leukemia cells through specific molecular pathways. For instance, if *S. sesban* constituents indeed inhibit Wnt/ β -catenin signaling (as in Abdelgawad's report), this could explain the reduced proliferation of leukemia cells in our treated rats, since Wnt signaling is crucial for leukemia stem cell maintenance (Siveen et al., 2017). Additionally, betulinic and oleanolic acids (found in *S. sesban* leaves; Mahamat et al., 2024) are known to induce mitochondrial apoptosis in cancer cells while being relatively non-toxic to normal cells (Mahamat et al., 2024). This property might underlie the selective action we observed: leukemia cells were cleared (lower WBC, lower blasts presumably), but normal bone marrow cells recovered (higher RBC and platelets).

Possible Mechanisms of Action

Based on the phytochemicals identified and the patterns of therapeutic effects, several mechanisms could be contributing to *S. sesban*'s action:

- **Antioxidant and Free Radical Scavenging:** High phenolic and flavonoid content in *S. sesban* likely provides strong antioxidant capacity. This can protect bone marrow and other tissues from oxidative stress caused by benzene metabolites and by the high metabolic activity of leukemia (Omeregic & Osagie, 2012). By reducing oxidative DNA damage, the extract may have slowed the progression of leukemic cell proliferation and prevented further mutations.
- **Induction of Apoptosis in Leukemic Cells:** Phytochemicals like saponins (e.g., chikusetsusaponin II, betulinic acid derivatives) present in *S. sesban* have demonstrated pro-apoptotic effects in leukemia cells (Abdelgawad et al., 2022). They may activate the intrinsic apoptosis pathway (mitochondrial) or extrinsic death receptor pathways in the leukemic blasts. The net effect would be a programmed cell death rather than necrosis, aligning with the low LDH output.
- **Cell Cycle Arrest:** Some flavonoids can cause cell cycle arrest at G₀/G₁ or G₂/M phase in cancer cells. If compounds in *S. sesban* (like the isolated flavone 3-hydroxy-4',7'-dimethoxyflavone; Dianhar et al., 2014) enact such an effect, the rampant division of leukemic cells would be curtailed, explaining the normalization of WBC counts.
- **Anti-inflammatory Pathways:** *S. sesban* extracts might inhibit key inflammatory mediators. The reduction in CRP implies less IL-6 activity; perhaps the extract suppressed NF- κ B or COX-2, as many plant polyphenols do, leading to lower cytokine production (Paszkievicz et al., 2012). Tannins, for example, can complex with pro-inflammatory proteins and neutralize them.

- **Immunomodulation:** It is possible that *S. sesban* enhanced the host's immune response to leukemia. Some plant extracts stimulate phagocytic activity or NK cell function (Akanni et al., 2012). While we did not directly measure immune parameters, the clearance of leukemia might have been aided by a boosted immune system in the extract-treated rats, akin to an "immunotherapeutic" contribution.
- **Protection of Organs:** Flavonoids like quercetin also stabilize endothelial function and protect organs like the heart and liver from toxins (Wolf & Baynes, 2006). Though not directly measured, *S. sesban* may have protected the liver (which produces CRP) and kidneys (excretion of waste) from leukemia or drug-related damage, thereby maintaining better systemic homeostasis.

Study Limitations

While our results are promising, several limitations should be acknowledged. First, the sample size (five rats per group) is relatively small, which is adequate for detecting large effects (as we did) but may not capture the full variability in responses. A larger cohort would strengthen confidence in the reproducibility of the findings. Second, our study focused on short-term outcomes (after 2 weeks of treatment). We did not assess long-term survival or whether the leukemia could relapse after stopping *S. sesban*. Future studies should include a follow-up period to examine if the herbal treatment provides a durable remission or if continuous administration is required to sustain the benefits. Third, we used a single mode of leukemia induction (benzene exposure) which primarily models a myeloid leukemia scenario. It remains to be seen if *S. sesban* extract would be equally effective in other leukemia models or subtypes (e.g., lymphoid leukemia, or genetically driven leukemia models). Fourth, we did not isolate which specific compounds in the crude extract are responsible for the observed effects. The extract is a complex mixture; pinpointing the active ingredient(s) would be valuable for standardization and could lead to the development of a defined drug. For now, we can only infer based on known constituents, but bioactivity-guided fractionation was beyond this study's scope. Lastly, while we observed no overt toxicity from *S. sesban*, a comprehensive toxicological assessment (including effects on non-target organs, biochemical indices for liver/kidney function, etc.) was not fully detailed here and should be performed to affirm safety, especially for higher doses or prolonged use.

Implications for Leukemia Therapy

Despite the above limitations, the implications of this work are notable. *Sesbania sesban* could serve as a basis for developing adjunct therapies in leukemia treatment. Its use alongside conventional chemotherapy might allow lower doses of chemo to be used, potentially reducing side effects while still achieving disease control – essentially acting as a chemo-sensitizer or protective co-therapy. An interesting observation from our data is that *S. sesban* addressed some issues that chemotherapy exacerbated (like anemia and LDH release). This opens up the possibility that combining the two could offset each other's

downsides: the plant extract can protect normal cells and ameliorate side effects, while chemo ensures any cancer cells not affected by the extract are killed. Some studies on natural product co-therapy in cancer support this idea (e.g., flavonoids protecting against doxorubicin's cardiotoxicity; Wolf & Baynes, 2006). Furthermore, for patients who cannot tolerate aggressive chemotherapy (such as the elderly or those with comorbidities), *S. sesban*-derived treatments might offer a gentler alternative or maintenance therapy to keep leukemia at bay. However, translating these findings to humans will require extensive further research.

Future Research Directions

Building on our results, we recommend several future avenues of investigation. First, isolating the most active anti-leukemic compounds in *S. sesban* and testing them individually can identify lead molecules for drug development. Compounds like oleanolic acid glycosides or the unique flavone identified by Dianhar et al. (2014) should be evaluated in vivo to see if they replicate the whole extract's efficacy. Second, studying the combination of *S. sesban* extract with standard chemotherapeutic agents in leukemic animals would be highly informative. Do they act synergistically or antagonistically? Our findings predict a beneficial synergy, but this should be confirmed by combination-index analyses and detailed bone marrow examinations. Third, mechanistic studies at the molecular level are needed. For instance, examining bone marrow histology and performing immunohistochemistry for markers of apoptosis (cleaved caspase-3), proliferation (Ki-67), and angiogenesis in treated vs untreated leukemia could help visualize *how* the extract works in the tissue context. Gene expression studies (via qPCR or RNA-seq) on sorted leukemia cells from treated animals could reveal up- or down-regulation of pathways like Wnt, NF- κ B, or p53, providing direct evidence of mechanism. Fourth, expanding to other models, including transgenic mice that develop leukemia or xenograft models of human leukemia, would take us closer to clinical relevance. If *S. sesban* shows efficacy in those models, it strengthens the case for eventual clinical trials. Lastly, any future use in humans would require ensuring the extract or its actives are standardized, safe, and can be administered in a controlled fashion. Pharmacokinetic studies in animals (to see how the compounds are absorbed, metabolized, and distributed) would be an important step in that direction.

Study Limitations and Strengths

In recognizing limitations, we also highlight that this study's strength lies in its integrative assessment – covering hematological, inflammatory, and enzymatic outcomes to provide a holistic picture of the treatment effect. We also directly compared the herbal treatment to conventional chemotherapy under the same conditions, which offers valuable perspective. The consistency between the biochemical markers and the blood counts adds internal validity to our data (e.g., low CRP and LDH coinciding with improved blood counts all point to true disease amelioration, not just a quirk in one type of measurement).

Moreover, our use of an established model of chemical leukemogenesis adds credibility, as this model has been used by other researchers and simulates many aspects of human leukemia pathology (Akanni et al., 2012). In conclusion, *Sesbania sesban* leaf extract has demonstrated a promising therapeutic profile in a rat model of leukemia: it significantly combats the leukemia itself while sparing the host from collateral damage. This dual benefit addresses a central challenge in cancer therapy and thus merits further exploration.

Conclusion

This study provides clear evidence that methanolic *Sesbania sesban* leaf extract exerts potent therapeutic effects in benzene-induced leukemic rats. The extract not only reduced the leukemic cell load – as shown by normalized white blood cell counts – but also ameliorated anemia and thrombocytopenia and dramatically lowered markers of inflammation (CRP) and tissue injury (LDH). In comparison to conventional chemotherapeutic treatment, *S. sesban* proved to be gentler on the organism, preventing the severe side effects (like extreme anemia and high tissue damage) that were observed with doxorubicin plus cytarabine. The bioactive compounds in *S. sesban* (phenolics, flavonoids, saponins, etc.) have demonstrated viability as effective anti-leukemic agents in this context. The highest dose tested (300 mg/kg) was particularly effective, bringing many parameters to levels indistinguishable from healthy controls. This indicates that *S. sesban* could emerge as a viable, organ-friendly adjunct or alternative for treating leukemia, due to its affordability, relative safety, and multi-faceted mechanism of action. In practical terms, our findings suggest that *S. sesban* leaf extract has the potential to be developed into a supportive therapy for leukemia patients, possibly reducing reliance on or doses of harsh chemotherapeutics. Its ability to destroy or suppress leukemia cells in vivo while simultaneously improving the body's resilience (less inflammation, less tissue damage) is a compelling advantage. Therefore, *S. sesban* represents a promising step toward more integrative and less toxic leukemia treatment strategies.

Recommendations

Building on these results, we recommend further research and development efforts as follows:

- **Mechanistic Studies:** Detailed investigations should be conducted to unravel the molecular pathways through which *S. sesban* extract affects leukemic cells and normal cells. Identifying specific cellular targets (e.g., signaling proteins or genes modulated by the extract) will help in understanding its mode of action and could guide optimization of therapy.
- **Isolation of Active Compounds:** The extract is a mixture of many compounds; isolating and testing the most active constituents (such as triterpenoid saponins or flavonoids identified in prior studies) is important. This could lead to the development of a purified drug derived from *S. sesban* with consistent potency.

- **Interaction with Conventional Chemotherapy:** Further studies should examine *S. sesban* extract in combination with standard chemotherapy drugs. It is crucial to determine whether the extract has any pharmacological interactions with drugs like doxorubicin or cytarabine – for example, does it interfere with their action or does it protect normal cells without shielding cancer cells? Such studies could inform combined therapy protocols.
- **Dose Optimization and Safety:** Different dosages and dosing schedules of *S. sesban* extract should be evaluated to find the optimal balance between efficacy and safety. Long-term toxicity studies (sub-chronic and chronic toxicity) in animals are also needed to ensure that even subtle adverse effects are ruled out. This is especially important if considering *S. sesban* for use in patients who might take it over extended periods.
- **Expand to Clinical Studies:** If further preclinical studies continue to yield positive results, preliminary clinical trials (phase I/II) could be designed to test *S. sesban* extracts or compounds in human leukemia patients, likely as an adjunct to existing therapy. Prior to that, ensuring quality control and reproducibility of the extract (or compound formulation) will be necessary.
- **Research on Synergy with Immunotherapy:** Since immunotherapy is an emerging pillar of cancer treatment (including CAR T-cell therapy and immune checkpoint inhibitors in leukemias), it would be interesting to investigate whether *S. sesban* could be combined with or complement immunotherapeutic approaches. Its immunomodulatory and anti-inflammatory effects might help mitigate cytokine release syndromes or other inflammatory side effects of immunotherapies.

In summary, *Sesbania sesban* has shown great promise in this study as a natural product with significant anti-leukemic and health-preserving properties. These findings lay a foundation for future work aimed at integrating such phytomedicine into leukemia care and improving outcomes for patients. With systematic research and clinical validation, *S. sesban* or its constituents could become part of innovative, multipronged strategies to treat leukemia in a safer and more holistic manner.

References

- Abdelgawad, A. M., Alharbi, S. A., & Al-Qahtani, M. A. (2022). Antiproliferative effects of saponin-rich compounds from *Sesbania sesban* leaves on leukemia cell lines. *Journal of Ethnopharmacology*, 287, 114926.
- Ajuru, M. G., Williams, L. F., & Ajuru, G. (2017). Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger Delta region of Nigeria. *Journal of Food and Nutrition Sciences*, 5(5), 198–205.
- Akanni, E. O., Alli, O. A. T., & Oloke, J. K. (2012). Anti-leukemic and immunomodulatory effects of fungal metabolites of *Pleurotus pulmonarius* and *P. ostreatus* on benzene-induced leukemia in Wistar rats. *Korean Journal of Hematology*, 47(1), 67–73.
- Alkhatib, A., & Alrakaf, M. (2019). Lactate dehydrogenase: Physiological roles and clinical implications. *American Journal of Biomedical Science and Research*, 3(5), 403–409.

- Asekun, O. T., Asekunowo, A. K., & Balogun, K. A. (2013). Proximate composition, elemental analysis, phytochemistry and antibacterial properties of the leaves of *Costus afer* Ker Gawl and *Cedrela odorata* L. from Nigeria. *Journal of Science Research*, 12(4), 101–108.
- Awad, A. B., & Fink, C. S. (2000). Phytosterols as anticancer dietary components: Evidence and mechanism of action. *Journal of Nutrition*, 130(9), 2127–2130.
- Berg, S., & Nand, S. (2017). Neurological complications of the leukemias across the ages. *Current Neurology and Neuroscience Reports*, 17(2), 13.
- Chandrawadan, V. P., Jadge, D. R., Nagoba, S. N., & Patil, S. S. (2021). Phytochemical study on *Sesbania sesban*: Isolated phytoconstituents for in-vivo anti-inflammatory and in-vitro antioxidant & anticancer activity. *Journal of Complementary Medicine Research*, 12(3), 101–118.
- Cooper, S. L., & Brown, P. A. (2015). Treatment of pediatric acute lymphoblastic leukemia. *Pediatric Clinics of North America*, 62(1), 61–73. <https://doi.org/10.1016/j.pcl.2014.09.006>
- Dianhar, H., Yuliana, R., & Fitriani, D. (2014). Inhibition of leukemia cells by flavonoids from *Sesbania sesban*. *Indonesian Journal of Cancer Chemoprevention*, 5(2), 82–88.
- Hwang, D., Kim, M., Park, H., & Jeong, M. (2019). Natural products and acute myeloid leukemia: A review highlighting mechanisms of action. *Nutrients*, 11(5), 1010. <https://doi.org/10.3390/nu11051010>
- Imaduddin, M. D., Saba, N., Eman, A., & Saba, K. (2022). Phytochemical screening and in vivo antileukemic activity of methanolic extract of seeds of *Nigella sativa* L. on benzene-induced leukemia in Wistar rats. *Asian Pacific Journal of Health Sciences*, 9(2), 194–198.
- Joshi, P. S., & Golgire, S. (2014). A study of salivary lactate dehydrogenase isoenzyme levels in patients with oral leukoplakia and squamous cell carcinoma by gel electrophoresis. *Journal of Oral and Maxillofacial Pathology*, 18(Suppl 1), S39–S44.
- Khalade, A., Jaakkola, M., Pukkala, E., & Jaakkola, J. (2010). Exposure to benzene at work and the risk of leukemia: A systematic review and meta-analysis. *Environmental Health*, 9(31), 1–8. <https://doi.org/10.1186/1476-069X-9-31>
- Kim, C., & Park, K. (2022). Association between phytochemical index and inflammation in Korean adults. *Antioxidants*, 11(2), 248.
- Lokesh, K., Kannabiran, J., & Rao, M. D. (2016). Salivary lactate dehydrogenase (LDH) – A novel technique in oral cancer detection and diagnosis. *Journal of Clinical and Diagnostic Research*, 10(2), ZC34–ZC37.
- Mahamat, O. B., Younes, S., Brahim, B. O., & Stevens, F. (2024). A review on medicinal and ethnomedicinal uses, biological features, and phytochemical constituents of *Sesbania sesban* (L.) Merr., a nitrogen-fixing plant native to the Republic of Chad. *The Scientific World Journal*, 2024, Article ID 1225999. <https://doi.org/10.1155/2024/1225999>
- Maher, T., Ahmad, R., Daddiouaissa, D., & Ahmad, F. (2021). Medicinal plants with anti-leukemic effects: A review. *Molecules*, 26(9), 2741. <https://doi.org/10.3390/molecules26092741>
- Ogbonnia, S. O., Nkemehule, F. E., & Anyika, E. N. (2009). Evaluation of acute and subchronic toxicity of *Stachytarpheta angustifolia* (Mill) Vahl (Verbenaceae) extract in animals. *African Journal of Biotechnology*, 8(9), 1793–1799.
- Ola, O. S. (2021). Anti-leukemogenic efficacy of Aloe vera gel on benzene-induced leukemia in Wistar rats. *Journal of Cancer and Tumor International*, 11(4), 38–49.
- Olufemi, A. E., Omotayo, O. I., David, A. B., & Monjeed, I. (2017). *Kigelia africana* stem bark, fruit and leaf extracts alleviate benzene-induced leukemia in rats. *Journal of Pharmaceutical Research International*, 18(2), 1–10.
- Omoriege, E. S., & Osagie, A. U. (2012). Antioxidant properties of methanolic extracts of some Nigerian plants on nutritionally stressed rats. *Nigerian Journal of Basic and Applied Science*, 20(1), 7–20.

- Ottaway, P. B. (2008). *Food Fortification and Supplementation – Technological, Safety and Regulatory Aspects*. CRC Press.
- Paszkiewicz, M., Budzyńska, A., Rozalska, B., & Sadowska, B. (2012). The immunomodulatory role of plant polyphenols. *Postępy Higieny i Medycyny Doswiadczałnej*, 66, 637–646.
- Potempa, L. A., Rajab, I. M., Hart, P. C., & Fernandez, P. M. (2021). C-reactive protein and cancer: Interpreting the differential bioactivities of its pentameric and monomeric, modified isoforms. *Frontiers in Immunology*, 12, 744129.
- Rao, A. V., & Koratkar, R. (1997). Anticarcinogenic effects of saponins and phytosterols. In G. R. Waller & K. Yamasaki (Eds.), *Saponins Used in Traditional and Modern Medicine. ACS Symposium Series* (Vol. 662, pp. 313–324). American Chemical Society.
- Siveen, K. S., Uddin, S., & Mohammad, R. M. (2017). Targeting acute myeloid leukemia stem cell signaling by natural products. *Molecular Cancer*, 16(1), 13. <https://doi.org/10.1186/s12943-016-0571-x>
- Watson, R. R., & Preedy, V. R. (2010). *Plant-Derived Antioxidants: Applications in Health and Nutrition*. CRC Press.
- Wolf, M. B., & Baynes, J. W. (2006). The anti-cancer drug, doxorubicin, causes oxidative stress-induced endothelial dysfunction. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1760(2), 267–271.