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Experimental Evaluation of the Hematopoietic and Immunomodulatory Effects of Phyllanthus amarus in Chloramphenicol-Induced Aplastic Anemia

K. Kaviya*[1], S. Swarnalatha[2], J. Karthi[3]

[1,2] *Department of Pharmacology, Pallavan Pharmacy College, Kanchipuram, Tamil Nadu, India*
[3] *Department of Pharmacognosy, Pallavan Pharmacy College, Kanchipuram, Tamil Nadu, India*

ABSTRACT

Aplastic anemia (AA) is a life-threatening hematological disorder characterized by bone marrow failure and peripheral pancytopenia, primarily mediated by immune dysfunction. The present study aimed to investigate the hematopoietic and immunomodulatory potential of the ethanolic extract of Phyllanthus amarus (EEPA) in a chloramphenicol-induced AA mouse model. Experimental mice were divided into six groups, including normal, model, low-, medium-, and high-dose EEPA (100, 200, and 400 mg/kg), and cyclosporine A (40 mg/kg) as a reference. Treatment with EEPA significantly restored hematological parameters such as white blood cells, red blood cells, hemoglobin, and platelet counts in a dose-dependent manner. Colony-forming assays revealed enhanced proliferation of erythroid, granulocyte–macrophage, and megakaryocytic progenitor cells. Flow cytometric analysis demonstrated immune restoration through increased $CD4^+$ and $CD4^+CD25^+FOXP3^+$ regulatory T-cell populations, with concomitant reduction in $CD8^+$ T cells. Western blot analysis confirmed downregulation of T-bet and upregulation of GATA-3 and FOXP3 proteins, indicating modulation of Th1/Th2/Treg balance. Histopathological evaluation of femoral and sternal bone marrow showed marked improvement in cellularity and reduction of adipose infiltration following EEPA administration. These findings suggest that Phyllanthus amarus exerts dual hematopoietic and immunoregulatory actions, supporting its potential as a safe and effective adjunct in the management of aplastic anemia.

Keywords: Phyllanthus amarus, Aplastic anemia, Hematopoiesis, Immunomodulation, Bone marrow, T-cell subsets, Chloramphenicol-induced toxicity.

INTRODUCTION

Aplastic anemia is a rare but serious hematological disorder characterized by bone marrow failure leading to peripheral pancytopenia. The condition arises due to the destruction or suppression of hematopoietic stem cells, resulting in decreased production of erythrocytes, leukocytes, and platelets (Young 2018). Patients typically present with symptoms such as anemia, frequent infections, and bleeding tendencies. Although the disease can be congenital or acquired, the acquired form is more common and often results from immune-mediated destruction of bone marrow stem cells. Activated cytotoxic T lymphocytes and inflammatory cytokines such as interferon-gamma and tumor necrosis factor-alpha play major roles in the pathogenesis by inducing apoptosis of hematopoietic cells and suppressing bone marrow regeneration. (Marsh et al 2009).

The incidence of acquired aplastic anemia varies globally, ranging from about two cases per million people per year in Western countries to as high as seven per million in Asian populations (Ahmed et al 2020). Higher prevalence in developing nations such as India has been associated with environmental pollutants, nutritional deficiencies, and infections (Moore et al 2023). The disorder may occur following exposure to certain drugs like chloramphenicol, carbamazepine, and cytotoxic agents, or to chemicals such as benzene and pesticides. Radiation exposure and viral infections are additional risk factors, although in nearly two-thirds of cases, the exact cause remains idiopathic (DeZern et al 2021).

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

At the cellular level, aplastic anemia involves immune dysregulation that triggers the activation of autoreactive cytotoxic T cells (Young et al 2006). These cells target hematopoietic progenitors, releasing cytokines that inhibit cell proliferation and induce apoptosis. Secondary factors such as stromal cell dysfunction, oxidative stress, and telomere shortening also contribute to disease progression (Scheinberg et al 2012). Genetic susceptibility involving mutations in genes like TERT and GATA2 may further predispose individuals to bone marrow failure or clonal evolution toward related disorders such as myelodysplastic syndrome or paroxysmal nocturnal hemoglobinuria (Kulasekararaj et al 2018).

Current therapeutic approaches include hematopoietic stem cell transplantation and immunosuppressive therapy. Stem cell transplantation offers a potential cure for young patients with suitable donors but is limited by donor availability, high cost, and complications such as graft-versus-host disease (Townsley et al 2014). Immunosuppressive regimens using antithymocyte globulin and cyclosporine can achieve satisfactory remission rates but are frequently associated with relapse, incomplete hematologic recovery, and risk of secondary malignancies (Killick et al 2016). Supportive measures like blood transfusions and iron chelation improve survival but do not address the underlying defect. These limitations highlight the urgent need for alternative or complementary strategies that are effective, affordable, and safe for long-term use.

In recent years, herbal medicines have gained increasing recognition due to their wide safety margin, cost-effectiveness, and multi-target therapeutic actions. Many medicinal plants possess antioxidant, anti-inflammatory, and immunomodulatory properties that can enhance hematopoiesis and reduce oxidative damage to bone marrow cells (Townsley et al 2014). Such natural compounds may help restore immune balance and protect against drug-induced or immune-mediated tissue injury, making them promising candidates for supportive therapy in bone marrow failure syndromes (Drexler et al 2020).

Phyllanthus amarus, a small herb belonging to the family Phyllanthaceae, has been widely used in traditional systems of medicine such as Ayurveda and Siddha (Oladele et al 2022). It is known for its hepatoprotective, antioxidant, and immunomodulatory effects, attributed to its rich content of lignans, flavonoids, alkaloids, and polyphenolic compounds (Rao et al 2021). Studies have reported that Phyllanthus amarus exhibits significant protective effects against oxidative stress and enhances hematological parameters in experimental models of toxicity. However, scientific evidence on its efficacy in bone marrow failure disorders like aplastic anemia is still limited.

Considering the limitations of existing therapeutic options and the pharmacological potential of Phyllanthus amarus, the present study has been designed to evaluate its hematopoietic, antioxidant, and immunomodulatory activities in experimental models of aplastic anemia. The outcome of this investigation may provide valuable preclinical evidence supporting the use of Phyllanthus amarus as a natural, safe, and economical adjunct in the management of aplastic anemia (Venkateswaran et al 2020).

MATERIALS AND METHODS

Plant Materials

Fresh leaves of Phyllanthus amarus were collected from the local areas of Kanchipuram district, Tamil Nadu. It was authenticated by Botanist Dr. K N Sunil Kumar of Siddha Central Research Institute, Arumbakkam, Chennai (Form No: PCOG002-ACF, code- P09092504A, Dated: 10/09/2025). The fresh samples were cleaned, shade dried and stored in standard condition.

Preparation of Ethanolic Extract of Phyllanthus amarus (EEPA)

Leaves of Phyllanthus amarus were collected, shade-dried at room temperature, and coarsely powdered. About 100 g of the powder was defatted with petroleum ether (60–80 °C) in a Soxhlet extractor for 6 h, followed by extraction with 95% ethanol (1:10 w/v) for 8 h until complete exhaustion. The extract were filtered and concentrated under reduced pressure below 40 °C, then dried in a vacuum oven to constant weight. The yield was calculated from the dry weight

of the leaves, and the final extract was stored in amber vials at 4 °C until use. The Soxhlet method enabled efficient extraction of lignans, flavonoids, alkaloids, and tannins responsible for the antioxidant and hepatoprotective activity of the plant (Bhat et al 2015).

Animals And Experimental Procedure

Male BALB/c mice (4–6 weeks of age and weighing 18–22g) were purchased. Mice were maintained in stainless steel cages under constant condition of temperature (23 ± 2·c), relative humidity (60 ± 2%) and lighting (12 h light/dark cycle). The animals had free access to water and food according to the Medical Experimental Animal Care guidelines.

All experimental procedures were carried out in strict accordance with the guidelines prescribed by the committee for the purpose of control and supervision on Experimentation on Animals and were approved by the Institutional Animal Ethics Committee (IAEC NO:2319/PO/Re/S/2025/CPCSEA.) from Pallavan pharmacy college, Kanchipuram.

Totally 36 mice were divided into 6 groups with 6 mice in each group: normal, model, low-, medium-, high-dose EEPA (100,200,400 mg/kg, L-, M-, H-EEPA), cyclosporine (CsA, 40 mg/kg). The experimental mice were orally treated with 130 mg.kg−1 BW of CMP for two weeks to induce acquired-aplastic anemia. After establishment of AA model, EEPA, CsA were intragastrically administered for 15 days at dosages abovementioned per day respectively.

Acute Oral Toxicity (Aot)Test

The acute oral toxicity of the ethanolic extract of *Phyllanthus amarus* (EEPA) leaves was evaluated following the method described by Lorke, (Lorke D 1983). The study was performed in two phases.

In the first phase, nine mice were randomly divided into three groups, each consisting of three animals. The groups received oral doses of 10, 100, and 1000 mg/kg body weight of the extract, respectively. All animals were closely observed for 24 hours for any signs of behavioral alterations, physical distress, or mortality.

In the second phase, four additional mice were divided into four groups comprising one animal each and administered higher doses of 1600, 2900, and 5000 mg/kg body weight of the extract orally. The animals were monitored continuously during the first 4 hours and periodically for 24 hours for any behavioral changes or mortality.

The absence of death or significant toxic signs at the highest tested dose was considered indicative of a wide margin of safety for the extract.

Assessment Of Body Weight, Food, Water Consumption, And Behavioral Changes

During the study, animals were monitored for body weight, food and water intake, and behavioral changes to assess their general health and response to *Phyllanthus amarus* treatment. Parameters such as activity, grooming, posture, and response to handling were recorded to detect signs of stress or toxicity, providing an overall indication of the extract's safety and therapeutic effect.

Peripheral Blood Count Analysis

Blood samples were collected from the tail vein of mice on days 0, 5, 10, and 15 following treatment. Hematological parameters, including total white blood cell (WBC) count, neutrophil (NEU) absolute value, platelet (PLT) count, red blood cell (RBC) count, and hemoglobin (Hb) concentration, were analyzed using an automated hematology analyzer (HORIBA ABX PENTRA XL 80, Montpellier, France).

Bone Marrow Pathological Examination

After 15 days of EEPA treatment, mice were euthanized by cervical dislocation, and femurs were carefully excised. One femur from each animal was fixed in picric acid for 24 hours, decalcified in 10% hydrochloric acid for 24 hours, and dehydrated through graded alcohols (50%–95%). The samples were then cleared in xylene, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). The prepared bone marrow sections were examined under a light microscope for histopathological evaluation.

Bone Marrow Semisolid Culture for Colony Forming Assay

Bone marrow nucleated cells were isolated from the femurs and cultured in a semisolid medium for the assessment of colony-forming units—erythroid (CFU-E), granulocyte-macrophage (CFU-GM), and megakaryocytic (CFU-MK). For CFU-E and CFU-GM assays, cells were plated in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 20% fetal bovine serum, 300 mg/L glutamine, 10 µg/L recombinant murine GM-CSF or erythropoietin (Epo), and 0.3% agar. For CFU-MK assays, cells were cultured in IMDM containing 20% fetal bovine serum, 300 mg/L glutamine, 1% bovine serum albumin, 10^{-5} mol/L 2-mercaptoethanol, and 10 µg/L recombinant murine thrombopoietin (Tpo). Each assay was performed in triplicate using 10^5 nucleated cells per well, incubated at 37 °C in a humidified 5% CO_2 atmosphere. Colonies of CFU-E (≥8 cells) and CFU-GM (≥40 cells) were counted after 5 days of incubation, while CFU-MK colonies (≥4 cells) were identified by acetylcholinesterase staining and counted after 7 days.

Analysis of T Lymphocyte Subpopulations by Flow Cytometry

After 15 days of treatment, blood samples were collected from the tail vein of each mouse and treated with 0.1% NH_4Cl to lyse red blood cells. The remaining leukocytes were stained with monoclonal antibodies against CD3-FITC, CD4-PE, and CD8-PE-Cy7 (eBioscience, USA), using corresponding isotype controls as negative references. Mean fluorescence intensity was determined using a flow cytometer (BD FACS Calibur, USA).

For splenic T-cell analysis, spleens were aseptically removed, minced, and homogenized to obtain single-cell suspensions. Red blood cells and debris were eliminated by sequential filtration through 70 µm and 40 µm cell strainers (BD Biosciences, USA). Regulatory T cells (Tregs) were quantified using a commercial Treg assay kit (eBioscience, USA), following the manufacturer's protocol. Fluorescence intensity was analyzed by flow cytometry to determine T-cell subpopulation distribution.

Western Blot Analysis

Following 15 days of EEPA administration, the spleens of mice from each experimental group were collected, finely minced, and homogenized using a tissue grinder. The spleen cell lysates were prepared according to standard protocols. Protein samples (30 µg) from each group were denatured by boiling and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The resolved proteins were then transferred onto nitrocellulose membranes (Amersham, USA). After blocking nonspecific binding sites, the membranes were probed with primary antibodies specific to T-bet, GATA-3, and FOXP3 (Santa Cruz Biotechnology, USA), followed by incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies (Santa Cruz Biotechnology, USA). The protein bands were detected using an enhanced chemiluminescence (ECL) detection kit with luminol substrate (Santa Cruz Biotechnology, USA). All experiments were independently conducted in triplicate to validate the reproducibility of the findings.

Pathological Observation of Sternum Marrow

Sternal bone marrow samples were collected and fixed in a suitable fixative, followed by decalcification and routine dehydration procedures. The processed tissues were embedded, sectioned, and stained with hematoxylin and eosin (H&E) to observe histopathological alterations in the bone marrow. The morphological changes were examined under a light microscope to assess the structural integrity and cellular composition of the marrow.

Statistical Analysis

All data are expressed as the mean ± standard deviation (SD). The statistical significance were analyzed by one-way analysis of variance (ANOVA), followed by post hoc multiple comparisons with Tukey's multiple comparison test using GraphPad Prism version 10.6. The differences observed were considered statistically significant at $p < 0.05$ or 0.01 (Jeon et al 2019).

RESULT AND DISCUSSION

EEPA Improved the General Condition of AA Mice

Mice in the normal group showed normal activity, appetite, and body weight. The model group exhibited reduced activity, poor appetite, dull eyes, weight, and hair loss. Treatment with medium and high doses of EEPA improved their condition, with mice appearing more active, eating well, and maintaining body weight.

Table 1. Effect of EEPA treatment on body weight, food and water intake, and behavioral observations in aplastic anemia mice.

TREATMENT	BODY WEIGHT	FOOD AND WATER INTAKE	BEHAVIORAL OBSERVATION
Normal	Stable	Adequate	Active, Healthy appearence
Model (Chloramphenicol 130mg/kg)	Decreased	Reduced	Lethargic, dull eyes, hair loss
L-EEPA (100 mg/kg)	Stable	Adequate	Active, normal grooming
M-EEPA (200 mg/kg)	Stable	Adequate	Increased activity Improved condition
H-EEPA (400 mg/kg)	Stable	Adequate	Highly active, restored normal appearance
csA 40mg/kg	Stable	Adequate	Active, comparable to normal group

EEPA Increased WBC, NEU, PLT, RBC Counts and Hb Concentration of AA Mice

Mice in the model group showed marked pancytopenia with significantly reduced WBC, NEU, RBC, PLT counts, and Hb levels compared to the normal group (all $P < 0.01$). EEPA treatment improved these hematological parameters in a dose-dependent manner. Medium- and high-dose EEPA groups showed significant increases in WBC, NEU, PLT, RBC, and Hb levels compared to the model group (all $P < 0.01$). CsA-treated mice also showed notable increases in these parameters ($P < 0.05$ or $P < 0.01$). These results indicate that EEPA enhances hematopoietic recovery in a dose-dependent manner.

Table 2 Effect of EEPA on hematological parameters in aplastic anemia mice

Group	Time	WBC (10^9/L)	NEU absolute value (10^9/L)	PLT (10^9/L)	RBC (10^{12}/L)	Hb (g/L)
Normal	Day 5	5.58±0.41	0.33±0.01	450±45.4	12.0±1.0	159.5±10.6
	Day 10	5.56±0.39	0.35±0.02	451±33	11.5±1.0	158.6±10.6
	Day 15	5.53±0.45	0.32±0.02	480±36.5	11.7±1.0	159.8±13.6
Model	Day 5	0.84±0.17*	0.03±0.01*	98.4±8.0*	7.5±0.8*	93.2±7.5*
	Day 10	0.85±0.13*	0.02±0.01*	90.6±6.48*	8.5±0.8*	90.2±8.5*
	Day 15	0.86±0.19*	0.02±0.01*	95.1±9.42*	8.5±0.9*	95.3±7.9*
L-EEPA	Day 5	1.18±0.17*	0.02±0.01	165±24.3*	8.1±0.7	105.4±8.3
	Day 10	1.51±0.28*	0.04±0.01*	175.7±22.3*	8.5±0.8	110.0±9.7*
	Day 15	1.64±0.28**	0.05±0.01*	175.5±25.5*	9.7±0.8*	125±10.4**
M-EEPA	Day 5	1.50±0.13*	0.05±0.01*	205.5±25.3**	8.9±0.6*	110.0±8.6*
	Day 10	1.75±0.83**	0.05±0.01*	255±22.2**	9.2±0.7*	109.0±8.6*
	Day 15	2.95±0.16***	0.9±0.01**	255.6±20.5**	10.2±1.0**	136±11.5**
H-EEPA	Day 5	2.1±0.11**	0.04±0.01	241±27.2**	8.8±0.7*	108.0±8.7*
	Day 10	2.56±0.35**	0.9±0.01**	299±29.4***	9.8±0.7**	123.5±8.7**
	Day 15	3.25±0.17***	0.15±0.02***	320±31.3***	10.3±1.1**	149±10.7***
CsA	Day 5	1.10±0.12*	0.02±0.01	115.2±23.5*	8.5±0.8	100.2±7.9
	Day 10	1.19±0.18*	0.04±0.01*	136±20.0*	8.3±0.8	115.0±9.1*
	Day 15	1.25±0.18**	0.04±0.01*	155.8±24.8*	9.5±1.0*	125.3±9.2**

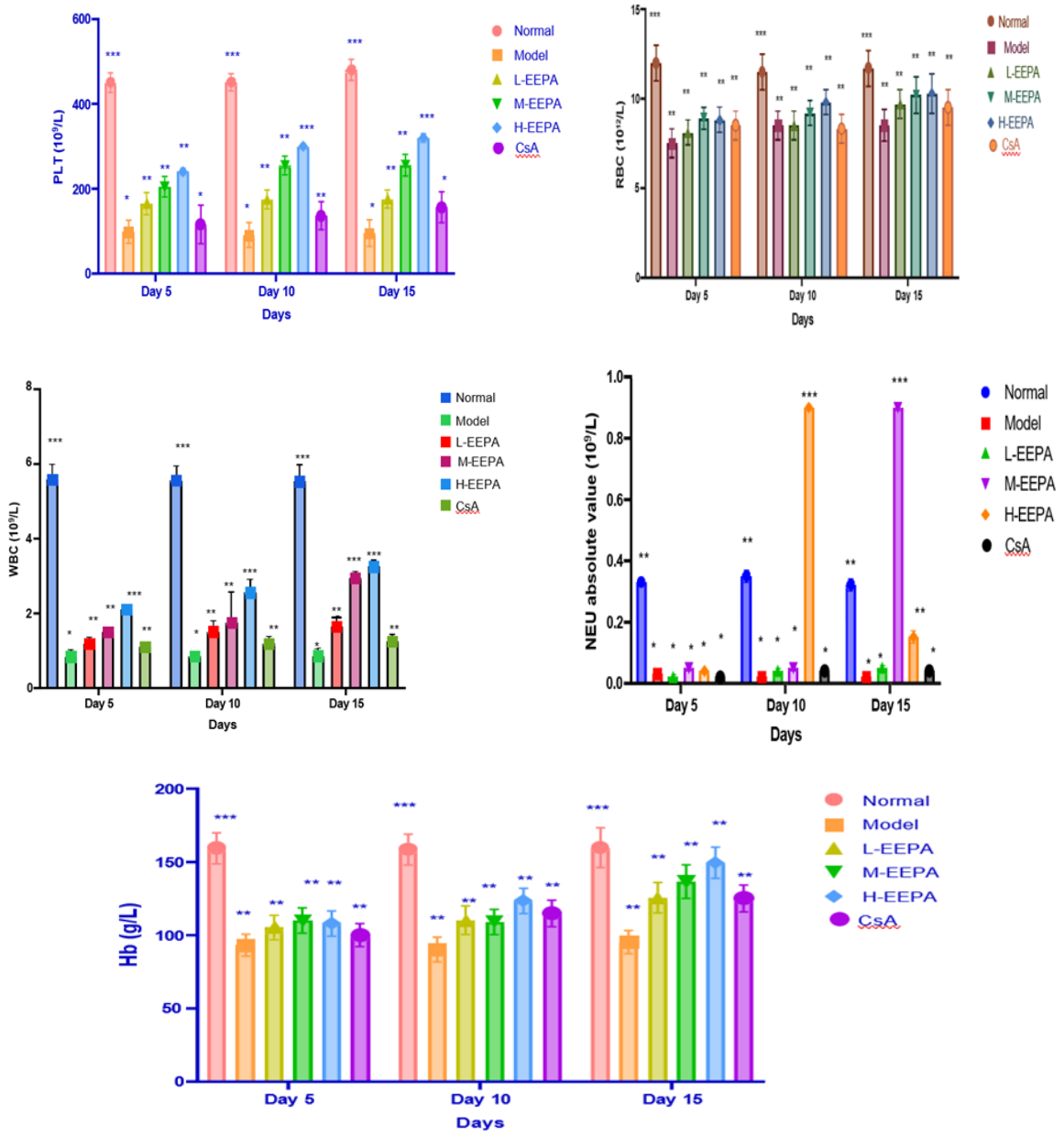


Fig.no.1 Effect of EEPA on peripheral blood parameters in aplastic anemia (AA) mice on days 5, 10, and 15. Parameters include WBC, NEU, PLT, RBC, and Hb across Normal, Model, L-, M-, and H-EEPA, and CsA groups. Values are mean ± SD (n = X). Statistical analysis by one-way ANOVA with Tukey's test; P < 0.05, *P < 0.01 vs. Model group.

EEPA Enhanced Proliferation of Hematopoietic Progenitor Cells in AA Mice

In vitro semisolid bone marrow culture revealed a marked reduction in CFU-E, CFU-GM, and CFU-MK colony numbers in the AA model group compared to normal controls. EEPA treatment significantly enhanced colony formation of all three progenitor types in a dose-dependent manner, with low-, medium-, and high-dose groups showing notable improvement over the model group ($P < 0.01$). Although CsA treatment also increased colony numbers, its effect was comparatively lower than that observed with medium- and high-dose EEPA, indicating a stronger hematopoietic stimulatory potential of EEPA.

Table 3 Effect of EEPA treatment on bone marrow colony-forming units (CFU-E, CFU-GM, and CFU-MK) in aplastic anemia mice.

Group	CFU-E (10^5 cells)	CFU-GM (10^5 cells)	CFU-MK (10^5 cells)
Normal	140.0±14.4	90.2±11.9	48±5.9
Model	22.8±2.5*	18.5±1.4*	11.1±2.1*
L-EEPA	34±3.0**	30.5±2.8**	19.8±2.1**
M-EEPA	42.6±2.4**	38.8±5.5**	25.8±3.6**
H-EEPA	63.5±3.5***	53.0±5.0***	38.5±5.6***
CsA	33.5±3.5**	29±2.7**	17.1±2.8**



Fig.no.2 Effect of EEPA on bone marrow CFU-E, CFU-GM, and CFU-MK in aplastic anemia mice. EEPA treatment significantly increased colony formation in a dose-dependent manner compared to the model group, with H-EEPA showing the highest effect. CsA also improved colony counts but less than M- and H-EEPA. Results are mean ± SD (n = 6). Statistical analysis by one-way ANOVA followed by Tukey's multiple comparison test; P < 0.05, P < 0.01, P < 0.001 vs. model group.

EEPA Altered Percentage of T Cells Subsets in AA Mice

Table 7.9 and Figures 3–4 illustrate the impact of EEPA on T cell subsets in peripheral blood and spleen of aplastic anemia (AA) mice. In the model group, $CD3^+$ and $CD3^+CD4^+$ cell percentages were markedly reduced, while $CD3^+CD8^+$ cells were significantly elevated compared with normal controls (all $P < 0.01$). EEPA treatment notably restored $CD3^+$ and $CD3^+CD4^+$ levels and reduced $CD3^+CD8^+$ cell percentages across all dose groups ($P < 0.05$ or $P < 0.01$). Similar immunoregulatory effects were observed in the CsA-treated group.

Additionally, splenic $CD4^+CD25^+FOXP3^+$ regulatory T cells (Tregs) were significantly decreased in model mice ($P < 0.05$). Treatment with medium- and high-dose EEPA significantly increased Treg levels toward near-normal values ($P < 0.05$), comparable to CsA. These findings suggest that EEPA effectively restores T cell subset balance, indicating its immunomodulatory potential in AA.

Table 4 Effect of EEPA treatment on T cell subsets in peripheral blood and spleen of aplastic anemia mice

Group	$CD3^+$	$CD3^+$ $CD4^+$	$CD3^+$ $CD8^+$	$CD4^+$ $CD25^+$	TREG
Normal	48.5±4.1	99.2±9.4	5.5±2.4	3.8±0.4	86.5±8.4
Model	19.9±1.7*	78.3±7.4**	23.8±2.0*	14.5±1.0*	75.5±6.8*
L-EEPA	32.5±3.1**	85.5±7.6**	9.0±0.8**	16.3±1.3**	81.8±7.8**
M-EEPA	34.1±3.1**	86.1±8.2**	8.3±0.6**	13.5±1.1**	85.4±7.9**
H-EEPA	41.2±3.6***	95.9±8.7***	5.5±0.4***	10.5±0.9***	85±8.6***
CsA	27.4±2.4**	95.8±8.9***	3.5±0.4***	6.2±0.4***	86.1±7.9***

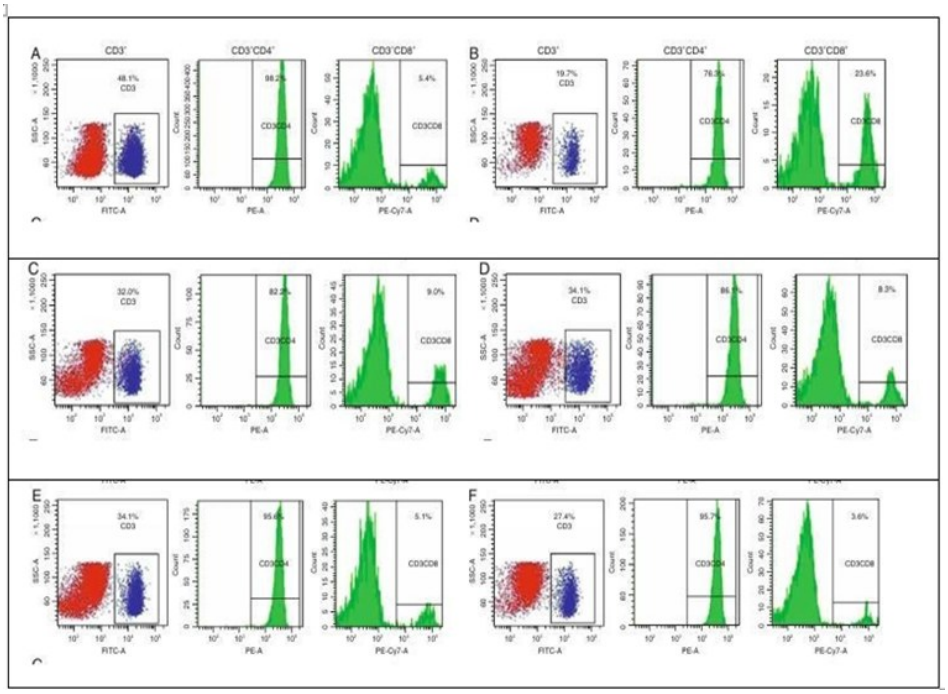


Fig.no.3 EEPA Altered the Percentage of T Lymphocyte Subset (%) of Peripheral Blood in AA Mice

Notes: A: Normal; B: Model; C: L-EEPA; D: M-EEPA; E: H-EEPA; F: CsA.

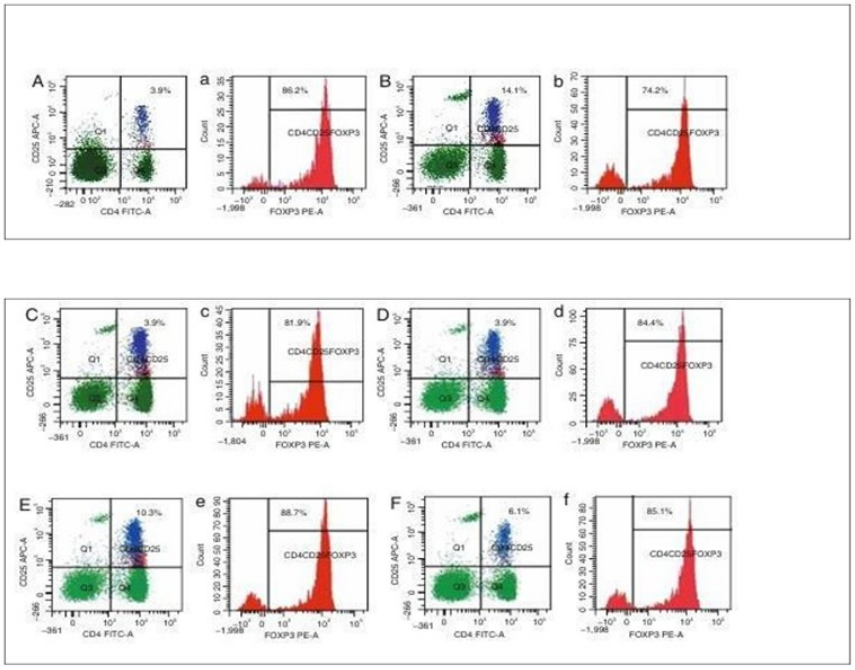


Fig.no.4 EEPA Increased the Percentage of Spleen CD4+CD25+FOXP3+ Cells in AA Mice

Notes: A,a: Normal; B,b: Model; C,c: L-EEPA; D,d: M- EEPA; E,e: H- EEPA; F,f: CsA.

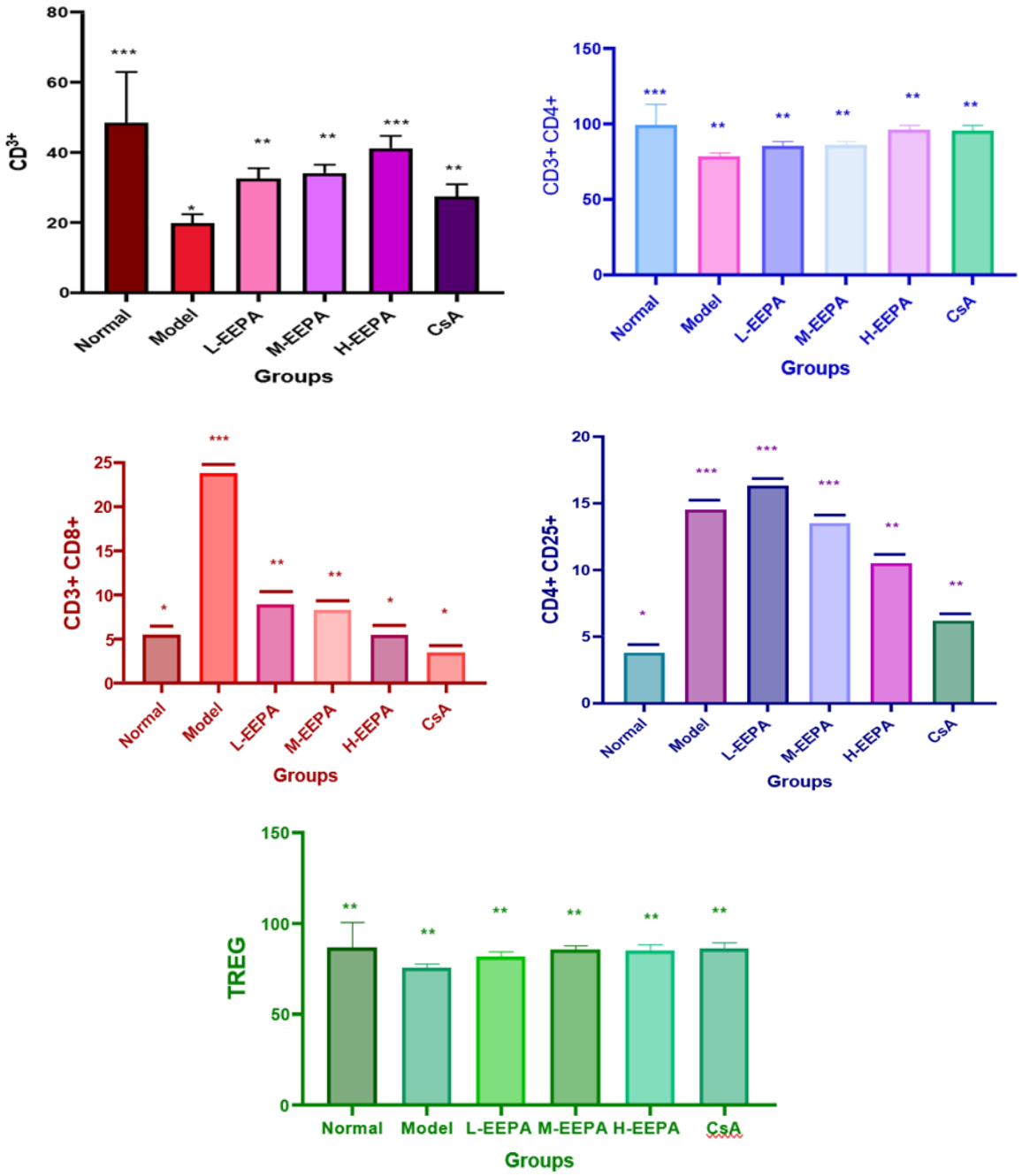


Fig.no.5 EEPA treatment significantly regulated T cell profiles in aplastic anemia mice.

The reduced percentages of $CD3^+$ and $CD3^+CD4^+$ cells in the model group were restored in the medium- and high-dose EEPA groups, while the elevated $CD3^+CD8^+$ cells were decreased toward normal levels. EEPA also increased splenic $CD4^+CD25^+FOXP3^+$ (Treg) cells, suggesting improved immune balance. The response was dose-dependent, with medium- and high-dose EEPA showing effects comparable to CsA. Data are expressed as mean ± SD (n = 6), analyzed by one-way ANOVA with Tukey's test (*P < 0.05, *P < 0.01 vs. model group).

EEPA reduced myelosuppression in AA mice

Histopathological analysis of bone marrow (Figure 6) demonstrated a marked improvement in hematopoietic recovery following EEPA treatment. In the normal control group, bone marrow sections exhibited typical hematopoietic hyperplasia with abundant myeloid, erythroid, and megakaryocytic cells and minimal adipose tissue, reflecting healthy marrow architecture. Conversely, the model group displayed severe myelosuppression characterized by a significant reduction in cellularity, depletion of

hematopoietic elements, and extensive adipose cell infiltration.

Administration of EEPA to AA mice significantly ameliorated these pathological alterations in a dose-dependent manner. Low-dose EEPA (L-EEPA) produced mild restoration of marrow cellularity, while medium- and high-dose groups (M-EEPA and H-EEPA) showed pronounced regeneration of hematopoietic cells across all lineages with a notable decline in adipose cell replacement. These findings indicate that EEPA effectively counteracted bone marrow suppression and enhanced hematopoietic recovery. In contrast, the CsA-treated group exhibited only minimal improvement in marrow morphology compared with the model group.

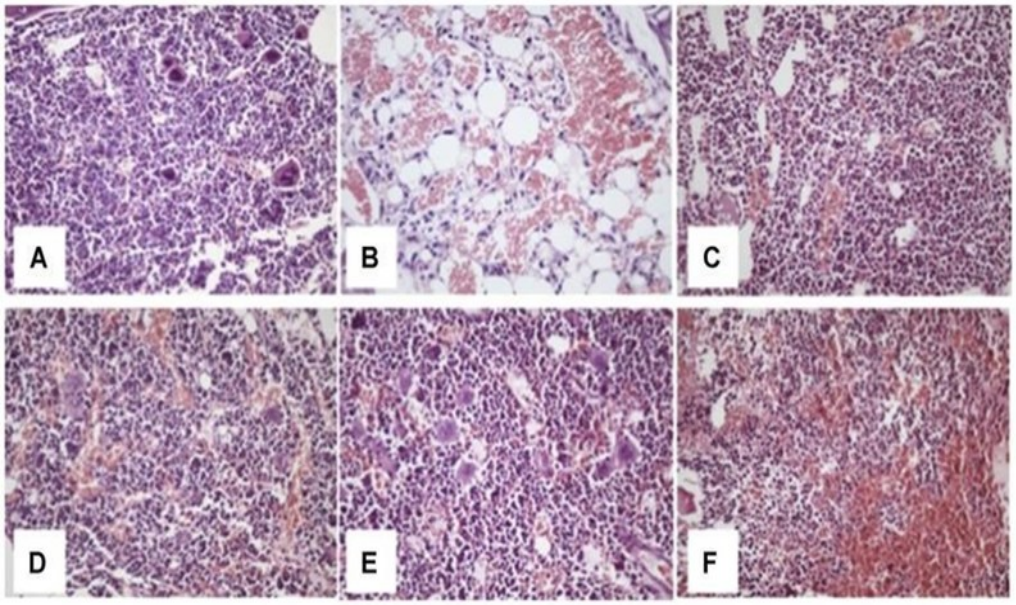


Fig.no.6 Effects of EEPA on bone marrow histology

The Wright-Giemsa-stained bone marrow sections were examined for the normal (A), control (B), EEPA low-dose (C), EEPA medium-dose (D), EEPA high-dose (E), and (F) CsA groups.

Pathological study on effects of sternum marrow

Histopathological examination of the sternum marrow revealed significant alterations among the experimental groups. In the normal control mice (Fig.7A), the sternum marrow displayed abundant hematopoietic tissue with well-developed blood sinusoids and minimal adipose content, indicating normal marrow activity. In contrast, the model group (Fig. 7B) exhibited severe marrow atrophy, characterized by marked depletion of hematopoietic elements, a drastic reduction in blood sinusoids, and extensive adipose tissue infiltration. The adipose stroma showed reticular cell proliferation accompanied by numerous lymphocytes and inflammatory infiltrates, with adipose tissue occupying approximately 60% of the marrow area, representing a significant increase compared with the normal group.

In the low-dose EEPA group (Fig. 7C), hematopoietic tissue density was reduced relative to the control but markedly improved compared with the model group. The blood sinusoids were more prominent, and adipose tissue content decreased to about 20%. The medium-dose (Fig. 7 D) and high-dose (Fig. 7 E) EEPA groups showed further restoration of marrow structure, with adipose tissue proportions reduced to approximately 25% and 38%, respectively, reflecting dose-dependent improvement.

In the CsA-treated group (Fig. 7 F), hematopoietic tissue showed moderate recovery compared to the model mice, with an average adipose tissue proportion of 45%. Although cellularity did not fully return to normal levels, CsA treatment partially improved marrow histoarchitecture and blood sinus formation.

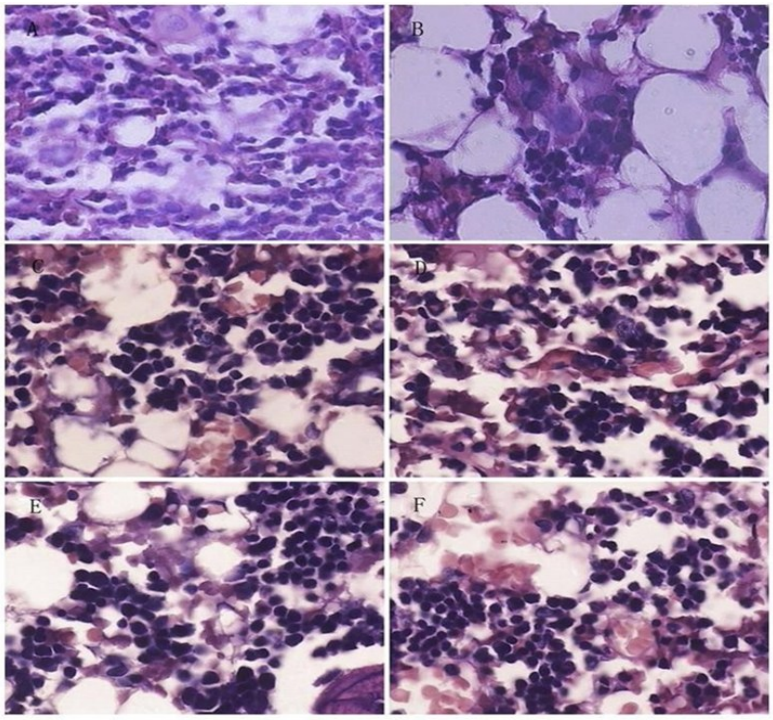


Fig.no.7 combined with thymus gland and thymus induced pathological changes in Blab/C mice. A- control group; B- model group; C- low-dose group; D- the mid-range dose;E- the high-dose group; F- CsA.

EEPA regulated T-BET, GATA-3 and FOXP3 proteins expression in AA Mice

Western blot analysis revealed that T-bet expression was significantly reduced in the spleen cells of mice treated with medium and high doses of EEPA compared to the model group ($P < 0.01$), similar to the CsA-treated group. In contrast, GATA-3 and FOXP3 expressions were notably increased in all EEPA-treated groups ($P < 0.05$ or $P < 0.01$), indicating EEPA's role in modulating immune-related transcription factors.

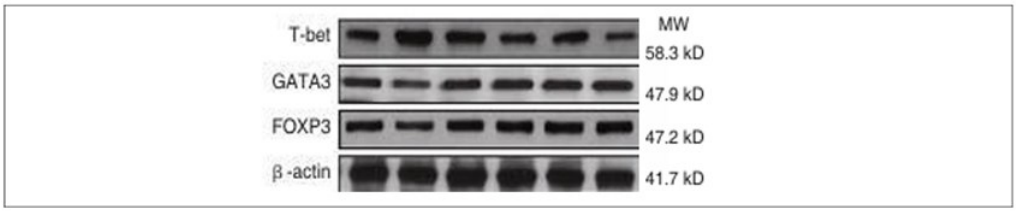


Fig.no.8 Effect of EEPA on T-bet, GATA-3, and FOXP3 protein expression in spleen cells of AA mice.

EEPA dose-dependently decreased T-bet and increased GATA-3 and FOXP3 expression compared to the model group. M- and H-EEPA showed effects similar to CsA. Values are mean ± SD ($n = 6$). Statistical analysis by one-way ANOVA followed by Tukey's test; $P < 0.05$, $P < 0.01$ vs. model group.

DISCUSSION

Aplastic anemia (AA) is a life-threatening hematological disorder characterized by bone marrow failure, leading to decreased production of all blood cell lineages. It is primarily caused by immune-mediated destruction of hematopoietic stem and progenitor cells by cytotoxic T lymphocytes. In AA, dysregulated immune responses result in an imbalance between helper ($CD4^+$) and cytotoxic ($CD8^+$) T cells, favoring excessive production of Th1 cytokines such as interferon-γ (IFN-γ), interleukin-2 (IL-2), and tumor necrosis factor-α (TNF-α). These cytokines suppress hematopoietic proliferation and induce apoptosis of bone marrow cells. Overexpression of the transcription factor T-bet drives Th1 differentiation and IFN-γ production,

further worsening bone marrow suppression. Meanwhile, regulatory T cells (Tregs), which maintain immune tolerance, are reduced in both number and function, leading to immune imbalance and progressive bone marrow failure.

In this study, administration of the ethanolic extract of Phyllanthus amarus (EEPA) significantly improved hematopoietic and immune parameters in an immune-mediated AA mouse model. EEPA dose-dependently increased peripheral white blood cell (WBC) counts, platelet (PLT) counts, and neutrophil (NEU) values. It also enhanced the colony-forming ability of bone marrow hematopoietic progenitor cells, including CFU-GM, CFU-E, and CFU-MK colonies. Histopathological analysis demonstrated that EEPA treatment effectively reversed myelosuppression, restoring normal marrow architecture with increased cellularity and reduced fatty infiltration. These findings suggest that EEPA enhances both proliferation and differentiation of hematopoietic stem and progenitor cells. The immunomodulatory effect of EEPA was further confirmed by the analysis of T-lymphocyte subsets. EEPA treatment significantly increased $CD4^+$ T cells and $CD4^+CD25^+FoxP3^+$ regulatory T cells, while reducing the percentage of $CD8^+$ T cells, thereby normalizing the $CD4^+/CD8^+$ ratio. Such modulation of T-cell populations indicates restoration of immune homeostasis and suppression of autoimmune-mediated marrow destruction. Western blot analysis of spleen tissue revealed that EEPA effectively regulated key transcription factors involved in T-cell differentiation. T-bet, a Th1-specific transcription factor, was markedly reduced in the medium- and high-dose EEPA groups, comparable to CsA-treated mice. In contrast, GATA-3, a Th2-associated transcription factor, and FoxP3, a marker of regulatory T cells, were significantly upregulated following EEPA treatment. These molecular changes suggest that EEPA shifts the immune response from a Th1-dominant pro-inflammatory state toward Th2 and Treg pathways, thereby promoting immune tolerance and bone marrow recovery. The comparative analysis showed that the effects of EEPA were similar to those of cyclosporine A (CsA), the standard immunosuppressive therapy used in AA. However, EEPA demonstrated an additional advantage by directly promoting hematopoiesis, whereas CsA primarily acts through immune suppression. The superior marrow regenerative response observed in EEPA-treated mice indicates that the extract may have growth factor–like activity, enhancing stem cell proliferation and differentiation while simultaneously correcting immune dysregulation. Collectively, these findings highlight the dual therapeutic action of EEPA—its ability to modulate immune responses and stimulate hematopoietic regeneration. By downregulating T-bet and upregulating GATA-3 and FoxP3 expression, EEPA restores immune balance and enhances bone marrow recovery. Therefore, Phyllanthus amarus may serve as a promising natural therapeutic candidate or adjunct to existing treatments like CsA in the management of aplastic anemia. Further studies involving isolation of active phytoconstituents and elucidation of molecular mechanisms could provide deeper insights into its pharmacological potential.

SUMMARY

The present study investigated the hematopoietic and immunomodulatory effects of Phyllanthus amarus in a chloramphenicol-induced aplastic anemia (AA) model in Wistar rats. Aplastic anemia, characterized by pancytopenia and bone marrow failure, was successfully induced using chloramphenicol. Treatment with Phyllanthus amarus extract resulted in significant improvements in hematological parameters, including red blood cells (RBCs), hemoglobin, white blood cells (WBCs), and platelets, compared to the disease model group. Colony-forming unit assays (CFU-E, CFU-GM, CFU-MK) demonstrated enhanced hematopoietic progenitor cell activity in extract- treated groups. Additionally, immunomodulatory assessment revealed a normalization of cytokine levels and improved immune cell function, indicating that the extract may modulate immune-mediated destruction of hematopoietic stem cells. These findings suggest that Phyllanthus amarus possesses both hematopoietic-stimulating and immunoregulatory properties.

CONCLUSION

Phyllanthus amarus exhibits promising hematopoietic and immunomodulatory effects in chloramphenicol-induced aplastic anemia. Its administration significantly restored blood parameters, enhanced

progenitor cell activity, and improved immune function, highlighting its potential as a therapeutic agent for AA. These results support further investigations, including mechanistic studies and clinical trials, to validate its efficacy and safety in the management of aplastic anemia.

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REFERENCE

1. Young NS. Aplastic anemia. N Engl J Med. 2018;379(17):1643–1656.
2. Marsh JC, Ball SE, Cavenagh J, et al. Guidelines for the diagnosis and management of aplastic anaemia. Br J Haematol. 2009;147(1):43–70.
3. Killick SB, Bown N, Cavenagh J, et al. Guidelines for the diagnosis and management of adult aplastic anaemia. Br J Haematol. 2016;172(2):187–207.
4. Young NS, Calado RT, Scheinberg P. Current concepts in the pathophysiology and treatment of aplastic anemia. Blood. 2006;108(8):2509–2519.
5. Risitano AM. Immunosuppressive therapies in aplastic anemia: where we stand and where we are going. Haematologica. 2022;107(7):1480–1493.
6. Townsley DM, Dumitriu B, Young NS. Bone marrow failure and the telomeropathies. Blood. 2014;124(18):2775–2783.
7. oshizato T, et al. Somatic mutations and clonal hematopoiesis in aplastic anemia. N Engl J Med. 2015;373:35–47.
8. Ahmed P, et al. Epidemiology of aplastic anemia: a study of 1,324 cases. Hematology. 2020;25(1):48–54. doi: 10.1080/16078454.2019.1711344
9. Montané E, et al. Epidemiology of aplastic anemia: a prospective multicenter study. Haematologica. 2008;93(4):518–523. doi: 10.3324/haematol.12213
10. Onishi Y, et al. Aplastic anemia: history and recent developments in diagnosis and management. Int J Hematol. 2024;119(1):15–29. doi: 10.1007/s12185-023-03584-9
11. Moore CA. Aplastic Anemia. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023. PMID: 32491548
12. Townsley DM, Dumitriu B, Young NS. Aplastic anemia: diagnosis and treatment. Mayo Clin Proc. 2014;89(11):1645–1659. doi: 10.1016/j.mayocp.2014.05.017
13. Malhotra P, Varma S, Marwaha RK, et al. High incidence of aplastic anemia is linked with lower socioeconomic status of Indian population. J Public Health (Oxf). 2016;38(2):223–228. doi: 10.1093/pubmed/fdv027
14. Peslak SA, Olson T, Babushok DV. Diagnosis and treatment of aplastic anemia. Curr Treat Options Oncol. 2017;18(12):70. doi: 10.1007/s11864-017-0516-y
15. Hosokawa K, et al. Hepatitis-associated aplastic anemia: epidemiology and clinical characteristics. Int J Hematol. 2017;106(3):359–364. doi: 10.1007/s12185-017-2265-9
16. Zhang J, et al. Environmental exposure and risk of aplastic anemia: a systematic review and meta-analysis. Hematology. 2019;24(1):1–9. doi: 10.1080/10245332.2018.1543175
17. Moore, C. A. (2023). Aplastic Anemia. StatPearls Publishing. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK534212/
18. Piekarska, A. (2024). The state of the art in the treatment of severe aplastic anemia. Frontiers in Immunology. Retrieved from https://www.frontiersin.org/articles/10.3389/fimmu.2024.1378432/full
19. Iftikhar, R. (2024). Evidence-Based Guidelines From the American Society for Transplantation and Cellular Therapy. ASTCT Journal. Retrieved from https://www.astctjournal.org/article/S2666-6367(24)00682-1/fulltext
20. Zhang, Y., et al. (2024). Peripheral blood stem cell transplantation vs. bone marrow transplantation in aplastic anemia: A systematic review and meta-analysis. PubMed. Retrieved from https://pubmed.ncbi.nlm.nih.gov/38076242/
21. Kulasekararaj, A., et al. (2024). A British Society for Haematology Guideline. PubMed. Retrieved from https://pubmed.ncbi.nlm.nih.gov/38247114/

22. Zhang, L., et al. (2024). Comparison of hematopoietic stem cell transplantation and immunosuppressive therapy in severe aplastic anemia. Frontiers in Immunology. Retrieved from https://www.frontiersin.org/articles/10.3389/fimmu.2024.1425076/full
23. Wang, L., et al. (2025). Efficacy and safety of immunosuppressive therapy versus alternative donor transplantation in severe aplastic anemia. Nature Reviews Hematology. Retrieved from https://www.nature.com/articles/s41408-025-01328-3
24. Drexler, B., et al. (2020). Very long-term follow-up of aplastic anemia treated with hematopoietic stem cell transplantation. Haematologica. Retrieved from https://pmc.ncbi.nlm.nih.gov/articles/PMC7536165/
25. Snyder, R. S., et al. (2020). Utilization & barriers to curative therapies among adult patients with aplastic anemia. Transfusion. Retrieved from https://www.sciencedirect.com/science/article/pii/S0006497118725743
26. Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Frontiers in Pharmacology, 4, 177. doi:10.3389/fphar.2013.00177
27. Posadzki, P., Watson, L., & Ernst, E. (2013). Adverse effects of herbal medicines: an overview of systematic reviews. Clinical Medicine, 13(1), 7–12. doi:10.7861/clinmedicine.13-1-7
28. Fabricant, D. S., & Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. Environmental Health Perspectives, 109(Suppl 1), 69–75. doi:10.1289/ehp.01109s169
29. Mahomoodally, M. F. (2013). Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. Evidence-Based Complementary and Alternative Medicine, 2013, 617459. doi:10.1155/2013/617459
30. Newman, D. J., & Cragg, G. M. (2016). Natural products as sources of new drugs from 1981 to 2014. Journal of Natural Products, 79(3), 629–661. doi:10.1021/acs.jnatprod.5b01055
31. World Health Organization (WHO). (2013). Traditional Medicine Strategy 2014–2023. Geneva: WHO. Retrieved from https://www.who.int/publications/i/item/9789241506090
32. Patel, J. R., Tripathi, P., Sharma, V., Chauhan, N. S., & Dixit, V. K. (2011). Phyllanthus amarus: Ethnomedicinal uses, phytochemistry and pharmacology: A review. Journal of Ethnopharmacology, 138(2), 286–313. https://doi.org/10.1016/j.jep.2011.09.040
33. Khatoon, A., Husain, I., Rather, I. A., & Jahan, N. (2020). Protective effect of Phyllanthus amarus against viral hepatitis: An updated review. Pharmacognosy Reviews, 14(28), 98–104. https://doi.org/10.4103/phrev.phrev_21_20
34. Venkateswaran, P. S., Iyer, S. V., & Kasinathan, S. (2020). Hepatoprotective activity of Phyllanthus amarus: A review update. Asian Journal of Pharmaceutical and Clinical Research, 13(3), 10–15. https://doi.org/10.22159/ajpcr.2020.v13i3.36550
35. Rao, K. V., Naidu, P. S., & Rao, T. R. (2021). Phytochemistry and hepatoprotective activity of Phyllanthus amarus: An updated review. International Journal of Research in Pharmaceutical Sciences, 12(2), 1389–1396. https://doi.org/10.26452/ijrps.v12i2.4672
36. Oladele, J. O., Ajayi, E. I., Oyewole, O. I., Oyeleke, O. M., Oladele, O. T., & Oladiji, A. T. (2022). Phytochemical, pharmacological, and toxicological properties of Phyllanthus amarus: A review. Scientific African, 15, e01095. https://doi.org/10.1016/j.sciaf.2021.e01095
37. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed., Vol II. Dehradun: International Book Distributors; 1987. p. 2181–2183.
38. Nadkarni KM. Indian Materia Medica. Vol I. Mumbai: Popular Prakashan; 2007. p. 961–963.
39. Warrier PK, Nambiar VPK, Ramankutty C. Indian Medicinal Plants: A Compendium of 500 Species. Vol 4. Chennai: Orient Longman; 1995. p. 324–327.
40. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. New York: Springer; 2007. p. 499–500.
41. Yadav NP, Dixit VK. Hepatoprotective activity of leaves of Phyllanthus amarus against carbon tetrachloride-induced liver damage in albino rats. Pharmaceutical Biology. 2003;41(4):263–268.

42. Patel JR, Tripathi P, Sharma V, Chauhan NS, Dixit VK. Phyllanthus amarus: Ethnomedicinal uses, phytochemistry and pharmacology. Pharmacogn Rev. 2011;5(10):120–123.
43. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. New York: Springer; 2007. p. 499–500.
44. Calixto JB, Santos AR, Filho VC, Yunes RA. A review of the plants of the genus Phyllanthus: their chemistry, pharmacology, and therapeutic potential. Med Res Rev. 1998;18(4):225–258.
45. Harish R, Shivanandappa T. Antioxidant activity and hepatoprotective potential of Phyllanthus amarus. Food Chem. 2006;95(2):180–185.
46. WHO. Monographs on Selected Medicinal Plants. Vol 2. Geneva: World Health Organization; 2002. p. 207–214.
47. Calixto JB, Santos AR, Filho VC, Yunes RA. A review of the plants of the genus Phyllanthus: their chemistry, pharmacology, and therapeutic potential. Med Res Rev. 1998;18(4):225–258.
48. Harish R, Shivanandappa T. Antioxidant activity and hepatoprotective potential of Phyllanthus amarus. Food Chem. 2006;95(2):180–185.
49. Yadav NP, Dixit VK. Hepatoprotective activity of leaves of Phyllanthus amarus against carbon tetrachloride-induced liver damage in albino rats. Pharm Biol. 2003;41(4):263–268.
50. Rajeshkumar NV, Joy KL, Kuttan G, Ramsewak RS, Nair MG, Kuttan R. Antitumour and anticarcinogenic activity of Phyllanthus amarus. J Ethnopharmacol. 2002;81(1):17–22.

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