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Histopatho-Protective Effects of the Aqueous Leaf Polyherbal Extract of in Phenylhydrazine-Induced Immune-Suppressed Rats

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Abstract

Phenylhydrazine (PHZ)-induced anemia can involve tissue injury in visceral and immune organs. This study investigated the histoprotective effects of a polyherbal aqueous leaf extract (PHALE) in PHZ-induced immunosuppressed Wistar rats. Plant samples were rinsed, shade-dried, pulverized, and processed into an aqueous extract. Thirty-six rats were divided into six groups of six animals each. Anemia was induced using PHZ at 40 mg/kg, and treatment groups received oral folic acid at 10 mg/kg or PHALE at 25, 50, or 100 mg/kg for 14 days. Liver, spleen, and thymus tissues were examined histologically on day 14. Extract treatment ameliorated PHZ-associated hepatocellular pyknosis, enhanced splenic lymphoid follicles and red pulp hematopoiesis, and preserved thymic cortical architecture. The most pronounced histological recovery occurred at 50–100 mg/kg. These findings suggest that PHALE has histoprotective potential in PHZ-induced anemia and associated immune alterations. The study provides preliminary histological evidence of protective effects on hepatic, splenic, and thymic tissues within this experimental model.

Keywords: Polyherbal Extract; Histoprotection; Phenylhydrazine; Anemia; Wistar Rats

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INTRODUCTION

When the immune system is compromised, the body becomes more susceptible to opportunistic infections and systemic disorders. Beyond increased infection risk, immunosuppression also has far-reaching effects on various physiological systems, including the hematopoietic system responsible for blood cell formation and oxygen transport (Kumar *et al.*, 2022; Patel *et al.*, 2025). One of the notable consequences and aggravating factors of immune dysfunction is anemia. It is characterized by reduced red blood cell count or haemoglobin concentration and can develop from chronic infections, nutritional deficiencies, or immune-mediated destruction of blood cells. It not only results from a weakened immune response but can also exacerbate immune compromise by impairing oxygen delivery to immune tissues and organs. This creates a vicious cycle, where immune weakness fuels anemia and anemia further suppresses immune efficiency (Nairz and Weiss, 2021). In particular, hemolytic anemia, caused by accelerated destruction of red blood cells, often involves immune and oxidative mechanisms that damage erythrocyte membranes. Addressing both immunosuppression and anaemia often requires interventions that support immune function, protect against oxidative damage, and enhance haematopoiesis. In recent years, plant-based immunomodulators have gained global attention as promising alternatives or adjuncts to synthetic drugs. Traditional medical systems, particularly in Africa and Asia, have long relied on herbal remedies to boost general body resistance, a concept now aligned with immunomodulation and immunoprotection (Patel *et al.*, 2025). Many plants are rich in bioactive phytochemicals such as flavonoids, alkaloids, saponins, and tannins, which can modulate immune function by increasing immune cell proliferation, enhancing antioxidant defenses, stimulating cytokine production, and improving red blood cell regeneration (Gasmi *et al.*, 2023; Kumar *et al.*, 2022). This study, therefore, aims to investigate histoprotective effects of the polyherbal aqueous leaf extract in phenylhydrazine-induced immunosuppressed rats.

MATERIALS AND METHODS

Plant Collection and Identification

Ficus capensis, *Justicia carnea,* and *Ipomoea batatas* are the species obtained from Egor Local Government Area of Edo State in Nigeria in June 2026. Prof. H. A. Akinnibosun identified and authenticated the plants in the Department of Plant Biology and

Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, with voucher numbers UBH-F331, UBH-J386, and UBH-I493, respectively (Ani *et al.,* 2020).

Extraction Preparation

Collected *Ficus sur*, *Justicia carnea,* and *Ipomoea batata* fresh leaves were rinsed in running water, shade-dry at room temperature. The dried samples were finely pulverized using an industrial grinder and kept in an airtight container. 93 g of each ground sample of the leaves (1:1:1) was dissolved in 1900 ml of distilled water in a soaking jar. The maceration was left to stand for 72 hours. The filtrate was concentrated in an oven, and a semi-solid extract was obtained (Obazelu and Williams, 2024).

Experimental Animals

A total of thirty-six (36) Wistar rats weighing 180 – 260 g were obtained from the Department of Biochemistry animal house, University of Benin. The animals were placed into plastic cages in the phytomedicine unit animal house. They were provided with free access to pelleted food and water for 14 days of acclimatization. The animal treatment complied with the guidelines for the use of laboratory animals (Ani *et al.,* 2020). It was approved by the ethical committee of Science Laboratory Technology with a reference number UNIBEN/ FSLT/ 00070

Anemia Induction of Wistar Rats.

Experimental design

After the establishment of anemia with 40 mg/kg phenylhydrazine, the weight of all the animals was measured, and the rats were randomly allocated to six groups of six rats each: Group 1: Anemic rats administered 25 mg/kg of the polyherbal extract. Group 2: Anemic rats administered 50 mg/kg of the polyherbal extract. Group 3: Anemic rats administered 100 mg/kg of the polyherbal extract. Group 4: Anemic rats administered 10 mg/kg folic acid (positive control). Group 5: Non-anemic rats, no treatment (normal control). Group 6: Untreated anemic rats (negative control). Gastric intubation was used to treat the rats orally for 14 days. The individual daily dose of the polyherbal extract for each rat in the treatment groups was calculated based on its most recent body weight using the formula: Dose (mg/kg) × Body Weight (g) / 1000 (Obazelu and Odionyenma 2024).

Histological Procedure

The organs were fixed in 10% formalin-saline and then dehydrated in four (4) concentrations of Isopropyl alcohol, i.e., 70, 80, 90, and 100 % during a 1 h period, then cleared in xylene and embedded in molten paraffin wax to eliminate the isopropyl alcohol. Tissues 5micrometer micro sections using a Leica RM 212 Rt. Rotary microtome sections were stained with Hematoxylin and Eosin (H+E) to show general tissue structure. Sectioned tissues were viewed and assessed under the Leica DM750 binocular microscope, complete with photomicrographic facilities, after which they were photomicrograph under the scope of a histopathologist. (Ahmed, 2016).

RESULTS

Plate 1 shows the histological features of the liver. In 25 mg/kg extract, liver showed distinct centriole, dilated sinusoidal. 50 mg/kg extract showed focal inflammation, mild steatosis while 100 mg/kg extract showed hydropic fatty changes, mild steatosis. In folic acid group, hepatocytes appear less distinct. Phenylhydrazine (PHZ) group showed pyknotic nuclei

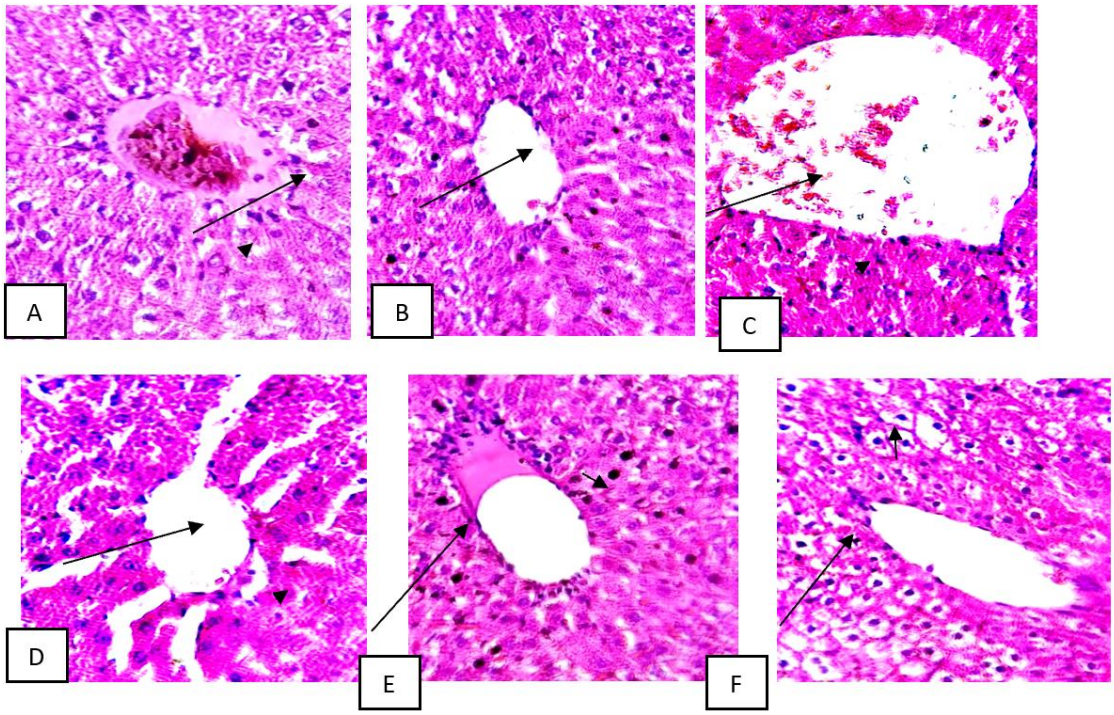


Plate 1: Effects of the polyherbal leave aqueous extract in the liver induced with phenylhydrazine hydrochloride

A. 40 mg/kg Phenylhydrazine Hydrochloride: Liver reveals visible centriole (long arrow) with the hepatocytes revealing pyknotic nucleus (short arrow).

B. Normal Control: liver reveals centriole surrounded by mild inflammatory cells (long arrow) with the hepatocytes revealing mild steatosis (short arrow).

C. 10 mg/kg Folic Acid: Liver reveals centriole (long arrow) surrounded by hepatocytes that appear not so distinct. (Short arrow).

D. 25 mg/kg Extract: Liver reveals distinct centriole (long arrow) with the hepatocytes and dilated sinusoidal (short arrow).

E. 50 mg/kg Extract: liver reveals centriole surrounded by focal inflammatory cells (long arrow) with the hepatocytes revealing mild steatosis (short arrow).

F. 100 mg/kg Extract: liver reveals centriole (long arrow) surrounded by hepatocytes with hydropic fatty changes and revealing mild steatosis (short arrow).

Plate 2 shows the histological features of the spleen. Lymphoid follicles are present in all groups, with varying degrees of activation. Red pulp appears distinct in the 40 mg/kg Phenylhydrazine Hydrochloride Group, coarse in the 50 mg/kg and 100 mg/kg extract, folic acid, and normal control group, and prominent in the 25 mg/kg extract. Blood Vessels are eccentrically located, with varying degrees of prominence.

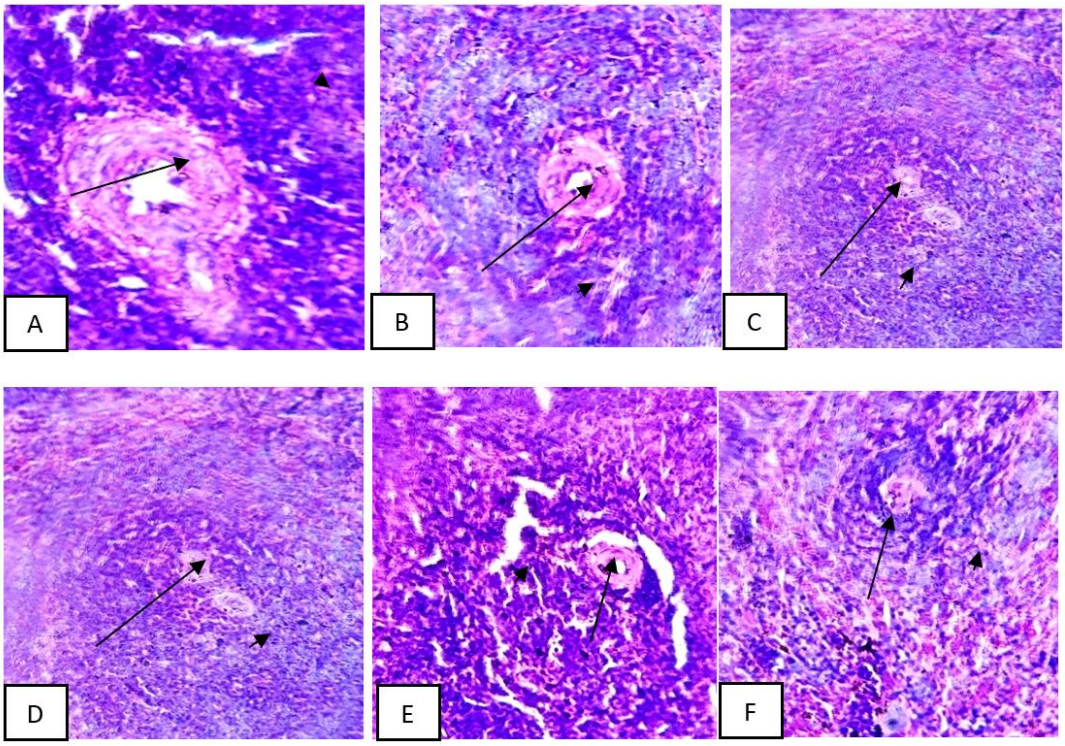


Plate 2: Effects of the polyherbal leaf aqueous extract in the spleen induced with phenylhydrazine hydrochloride

A. 40 mg/kg Phenylhydrazine Hydrochloride: spleen shows lymphoid follicles (short arrow) with centrally to eccentrically located large blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes. The red pulp appears distinct.

B. **Normal Control:** spleen shows lymphoid follicles (short arrow) with centrally to eccentrically located large blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes. The red pulp appears coarse.

C. **10 mg/kg Folic Acid**: spleen shows lymphoid follicles (short arrow) with eccentrically located blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes which appear activated. The red pulps appear coarse.

D. **25 mg/kg Extract**: spleen shows prominent lymphoid follicles (short arrow) with eccentrically located blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes. The red pulp is prominent.

E. **50 mg/kg Extract**: spleen shows lymphoid follicles (short arrow) with not so prominent eccentrically located blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes. The red pulp appeared coarse.

F. **100 mg/kg Extract**: spleen shows lymphoid follicles (short arrow) with eccentrically located blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes which appear activated. The red pulp appears coarse

Plate 3 shows the histological features of the thymus. 25 mg/kg extract and normal control showed the presence of cortex containing vacuolated epithelial cells and mononuclear cells in the medullary adipose tissue. There was reduced adipose tissue in the thymus of groups treated with 50 mg/kg and 100 mg/kg extract. Comparable thymus morphology between the 100 mg/kg extract group and the normal control group.

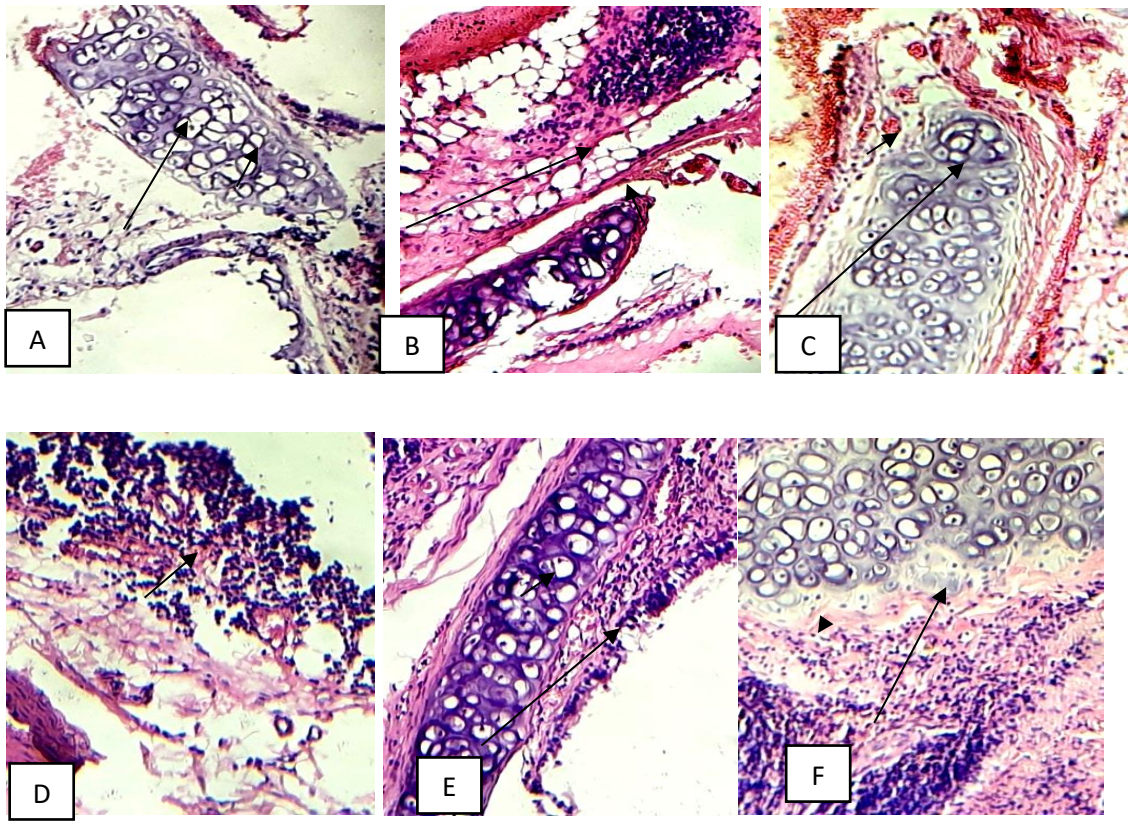


Plate 3: Effects of the polyherbal leaf aqueous extract in the thymus induced with phenylhydrazine hydrochloride

A. Normal Control: Thymus revealed cortex containing vacuolated epithelial cells (long arrow) and mononuclear cells in the medullary adipose tissue (short arrow).

B. 10 mg/kg Folic Acid: Thymus revealed cortex surrounding (long arrow) adipose tissue (short arrow) and medulla.

C. 40 mg/kg Phenylhydrazine Hydrochloride: Thymus revealed cortex surrounding (long arrow) adipose tissue (short arrow) and medulla.

D. 25 mg/kg Extract: Thymus revealed cortex containing vacuolated epithelial cells (long arrow) and diffuse mononuclear cells in the medullary adipose tissue (short arrow).

E. 50 mg/kg Extract: Thymus revealed cortex surrounding (long arrow) adipose tissue (short arrow) and slight mononuclear cells in the medulla.

F. 100 mg/kg Extract: Thymus revealed cortex surrounding (long arrow) adipose tissue (short arrow) and medulla.

DISCUSSION

The histology outcome of liver in plate 1 was that in 25 mg/kg extract, liver revealed distinct centriole, dilated sinusoidal. 50 mg/kg extract showed focal inflammation and mild steatosis while 100 mg/kg extract showed hydropic fatty changes, mild steatosis. In folic acid group, hepatocytes appear less distinct. The phenylhydrazine (PHZ) group showed pyknotic nuclei, which indicate liver damage or stress. The extract Groups showed Varying degrees of improvement (distinct centriole, mild steatosis), which indicate potential hepatoprotective effects. This is in line with the study by Josiah et al. (2024), who found that research done on *Ficus capensis* showed hepatoprotective activity via antioxidant and anti-inflammatory effect. On the same note, Ukpabi-Ugo et al. (2019) reported that the histopathological analysis showed that *Justicia carnea* contained hepatoprotective properties because the extract bettered liver structure and alleviated necrosis in the treated rats, in relation to CCl_4 administration only. Along with this, Adebayo et al. (2020), who showed that *Moringa oleifera* leaf extract had high anti-anemic and hepatoprotective effects on phenylhydrazine-induced anemic Wistar rats, ascribed its effects to antioxidant and anti-inflammatory activity. In addition, Sheth et al. (2021) discovered that the polyherbal formulation of *Raktavardhak Kadha* (RK) enhanced histoprotective parameters and alleviated oxidative stress in anemic rats, suggesting the possible hepatoprotective effects. Histopathological evidence (pyknotic nuclei, steatosis) indicates liver damage or liver stress; this indicates PHZ toxicity. According to Ramirez et al. (2018), acute liver injury is caused by exposure to chemicals, drugs, or viral infection. The outcome of chronic liver disease is normocytic and macrocytic anemias (Allahmoradi *et al.,* 2020). The Possible mechanism of the polyherbal extract's protective effect on the liver is via antioxidant activity, anti-inflammatory effects, and the presence of bioactive compounds such as flavonoids and phenolics, which assist liver performance.

The histological outcome of the spleen in Plate 2 indicated that lymphoid follicles are present in all groups, with varying degrees of activation. Lymphoid follicles are prominent in the 25 mg/kg extract group. The follicles (white pulp) appeared activated in folic acid and 100 mg/ kg of the polyherbal aqueous extract. Lymphoid follicles are clusters of immune cells, including B cells and T cells, that are found in lymphoid organs such as the spleen and lymph nodes. When these follicles are activated, they produce antibodies and other immune molecules that help to fight off infections and diseases. The activation

of lymphoid follicles is a sign of a healthy immune response to the phenylhydrazine-induced damage, and it suggests that the polyherbal extract may have immunomodulatory effects. Red Pulp appeared distinct in the phenylhydrazine hydrochloride group, coarse in the normal control group, folic acid group, 50 and 100 mg/kg extract group, and prominent in the 25 mg/kg extract group. The white pulp is involved in the immune response, producing antibodies and activating immune cells to fight infections, while the red pulp filters the blood and removes old or damaged red blood cells. Blood vessels, eccentrically located, appeared large in phenylhydrazine and normal control groups. The polyherbal aqueous leaf extract appears to have a protective effect on the spleen of treated rats, and may involve modulation of the immune system. This finding is in line with the study of Gupta et al. (2018), who reported that polyherbal extracts can modulate the immune system by affecting the production of cytokines and the activation of immune cells. Another study on *Ficus capensis Thunb.* (Moraceae) found out that it had haematoprotective effects on phenylhydrazine-induced anaemic rats (Ezeigwe et al., 2020). Likewise, Rao et al. (2020) noted that *Moringa oleifera* Lam. (Moringaceae) exerted a protective action on hematology parameters in the rats subjected to gamma radiation. Possible mechanism of the polyherbal extract's protective effect on the spleen is through antioxidant activity, anti-inflammatory effects and immune modulation.

The histological result of the thymus in Plate 3 showed promising results. The extract appears to have a protective effect on the thymus, with varying doses exhibiting different levels of mononuclear cell infiltration and adipose tissue presence. In extract-treated groups (25, 50 and 100 mg/kg), the thymus showed improved histological features, with reduced mononuclear cell infiltration and preserved cortex and medulla architecture. Similarly, in the normal control group, the thymus showed a cortex with vacuolated epithelial cells and mononuclear cells in medullary adipose tissue. In the Phenylhydrazine Hydrochloride Group, the thymus showed a cortex surrounding adipose tissue and medulla, indicating potential damage. The histopathological results suggest that the extract may have protected the thymus gland, as evidenced by the presence of cortex containing vacuolated epithelial cells and mononuclear cells in the medullary adipose tissue. The thymus is a critical organ for the development and maturation of T-lymphocytes (T-cells), which are essential for cell-mediated immunity. The cortex is where T cell precursors undergo selection and maturation, while the medulla is involved in the final stages of T cell maturation and the elimination of autoreactive T cells. This observation is consistent with

the research work by Oyewole et al. (2022) who discovered that polyherbal extract possessed antioxidant and immunomodulatory properties against the anemic rats that were induced by phenylhydrazine. Equally, Ezeigwe et al. (2020) found that bioactive compounds contained in extract, including flavonoids and phenolic acid, could have enhanced thymus regeneration. It has been presented those different substances, such as herbal extracts, can influence the structure and work of the thymus. Some studies have stated that some herbal extracts can stimulate the immune performance by stimulating the growth of T cells or a change in the immune cells' ratio in the thymus (Liu et al., 2019; Zhang et al., 2020). Possible mechanism of the polyherbal extract protective effect on the thymus is through antioxidant activity, anti-inflammatory effects and presences of bioactive compounds such as flavonoids, phenolics supporting thymus function.

CONCLUSION

This study suggests that polyherbal aqueous leaf extract (PHALE) has histoprotective potential in phenylhydrazine-treated Wistar rats. Following 14 days of treatment, histological observations indicated partial improvement in hepatic morphology, changes in splenic lymphoid follicles and red pulp, and preservation of thymic cortical and medullary architecture. However, responses varied across doses and organs, and persistent hepatic inflammation, steatosis, and hydropic changes indicate that tissue protection was incomplete.

These findings contribute preliminary preclinical evidence concerning the effects of PHALE on hepatic, splenic, and thymic tissues in an experimental model of phenylhydrazine-induced anemia. The observed structural changes support further investigation of its protective potential, although their functional significance and underlying mechanisms remain to be established.

Further studies should incorporate quantitative histopathological assessment alongside biochemical and functional measurements to determine whether the observed tissue changes correspond to improved organ function. Larger samples and longer observation periods are also needed to clarify dose-related responses, evaluate safety, and establish the reproducibility of these findings before therapeutic applications can be considered.

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