Keywords
Musa paradisiaca spathe; Iron deficiency anemia; Wound healing; Toxicity evaluation.

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Comprehensive Preclinical Evaluation of *Musa Paradisiaca* Spathe Extract: Phytochemical Characterization, Safety Assessment, Wound Healing Activity, and Anti-Anemic Efficacy

Abstract

Musa paradisiaca spathe, an underutilized agricultural by-product, has emerged as a promising source of bioactive compounds with potential nutraceutical applications. This study systematically investigated the phytochemical composition, safety, wound-healing activity, and anti-anemic efficacy of hydroethanolic *M. paradisiaca* spathe extract (HAE-MP). Aqueous, ethanolic, and hydroethanolic extracts were characterized using ICP–MS and GC–MS analyses. The hydroethanolic extract exhibited the highest extraction yield (21.11%), greatest iron content (883.18 mg/kg), and the most diverse phytochemical profile. In vitro scratch assay and RT-qPCR analysis demonstrated accelerated wound closure, accompanied by downregulation of the pro-inflammatory cytokines IL-6 and TNF-α and restoration of IL-10 expression, indicating enhanced tissue repair. Safety was evaluated according to OECD Guidelines 423 and 407, revealing no mortality, treatment-related clinical signs, hematological or biochemical abnormalities, or histopathological changes following acute and repeated-dose oral administration. Anti-anemic efficacy was assessed in a phenylhydrazine-induced hemolytic anemia model in Wistar rats. Oral administration of HAE-MP (500 mg/kg) significantly improved erythrocyte count, hemoglobin, hematocrit, serum iron, ferritin, and transferrin saturation while reducing total iron-binding capacity, erythropoietin, reticulocyte count, and oxidative stress biomarkers. Although ferrous ascorbate produced a greater therapeutic response, HAE-MP demonstrated substantial hematological recovery with an excellent safety profile. These findings suggest that the hydroethanolic extract of *M. paradisiaca* spathe possesses significant wound-healing and hematinic properties, highlighting its potential as a sustainable botanical resource for future nutraceutical and phytopharmaceutical development.

INTRODUCTION

Iron deficiency anaemia (IDA) is the most common nutritional disorder globally, affecting nearly two billion people and posing a major public health challenge, particularly in low- and middle-income countries. The disorder arises from insufficient iron availability for haemoglobin synthesis, leading to reduced oxygen transport, fatigue, impaired cognitive performance, decreased work capacity, and increased vulnerability to infections (Kassebaum et al., 2014). Although oral iron supplementation remains the standard treatment, its effectiveness is often limited by gastrointestinal side effects, poor patient adherence, and inconsistent bioavailability, emphasizing the need for safer and more effective plant-based therapeutic alternatives (Camaschella, 2015).

Medicinal plants have long served as important sources of bioactive compounds for the prevention and management of haematological disorders. Botanical extracts enriched with phenolics, flavonoids, tannins, and other phytoconstituents have gained increasing attention because of their antioxidant and erythropoietic properties. Since oxidative stress contributes significantly to erythrocyte damage and impaired iron metabolism, phytochemicals capable of neutralizing reactive oxygen species may facilitate haematological recovery while protecting cellular integrity (Vasconcelos et al., 2018).

M. paradisiaca L. (Musaceae), commonly known as banana or plantain, is one of the world's most extensively cultivated tropical crops and possesses considerable nutritional, medicinal, and economic importance. Traditionally, its fruits, peels, pseudostems, flowers, leaves, and other plant parts have been used for treating gastrointestinal disorders, diabetes, inflammatory conditions, wound healing, and microbial infections (Pereira and Maraschin, 2015). Phytochemical investigations have identified a diverse range of bioactive constituents, including polyphenols, flavonoids, alkaloids, sterols, and fatty acids, which collectively contribute to its broad pharmacological profile (Mathew and Negi, 2017).

Among these plant components, the banana spathe (inflorescence bract) remains one of the least explored despite being produced in large quantities during cultivation and generally discarded as agricultural waste. Available evidence indicates that banana floral tissues are rich in essential minerals, dietary fibre, phenolic compounds, and antioxidant metabolites with promising nutraceutical potential (Bennett et al., 2010). Their nutritional richness and abundance suggest that banana spathe could serve as a sustainable botanical resource capable of promoting haematological health while simultaneously supporting agricultural waste valorization.

In addition to its nutritional value, *M. paradisiaca* exhibits strong antioxidant activity attributed primarily to its phenolic constituents. Extracts from various banana tissues have demonstrated potent free-radical scavenging, cytoprotective, and anti-inflammatory effects, indicating their potential to alleviate oxidative stress-related disorders (González-Montelongo et al., 2010). Given the central role of oxidative stress in erythrocyte destruction and the progression of anaemia, phytochemically rich banana spathe extracts may offer a multifactorial strategy for restoring haematological function and improving iron homeostasis.

Despite growing evidence supporting the biological activities of *M. paradisiaca*, comprehensive studies integrating phytochemical characterization, toxicological safety assessment, and anti-anaemic efficacy evaluation of banana spathe extracts remain limited. Furthermore, the relationship between extract composition, safety profile, and hematopoietic activity has not been adequately explored. Establishing such evidence is essential for the development of scientifically validated phytopharmaceuticals and nutraceutical products derived from this underutilized plant resource.

Therefore, the present study aimed to comprehensively evaluate a hydroethanolic extract of *M. paradisiaca* spathe through phytochemical screening and chromatographic characterization, followed by assessment of its biological activity in an in vitro scratch assay, acute oral toxicity study conducted according to OECD Guideline 423, repeated-dose toxicity evaluation according to OECD Guideline 407, and anti-anaemic efficacy in an experimentally induced rat model. The present study aimed to evaluate the safety and therapeutic efficacy of *M. paradisiaca* spathe as a sustainable botanical resource for the management of iron deficiency-associated haematological disorders.

2. Materials and Methods

2.1 Plant Material Collection and Preparation

Fresh spathes of *M. paradisiaca* L. were harvested from cultivated banana plants in West Bengal, India. The collected material was rinsed thoroughly with distilled water to remove soil and other extraneous matter, followed by shade drying at room temperature until a constant weight was attained. The dried spathes were then milled using a mechanical grinder, and the resulting powder was sieved through a fine mesh to obtain a uniform particle size for subsequent extraction procedures.

2.2 Preparation of Extracts

Three solvent extracts of *M. paradisiaca* spathe were prepared using aqueous, ethanolic, and hydroethanolic extraction systems. Briefly, 0.5 g of the powdered spathe was suspended in 10 mL of the respective solvent: distilled water (aqueous extract, AqE), absolute ethanol (ethanolic extract, EtE), or ethanol–water (50:50, v/v; hydroethanolic extract, HAE-MP). The suspensions were subjected to ultrasonic-assisted extraction for 30 min at room temperature to enhance the recovery of soluble phytoconstituents. Following extraction, each sample was filtered through a 0.45 µm membrane filter, and the filtrates were stored at 4°C until subsequent physicochemical and phytochemical analyses.

The extraction yield was calculated as:

$$Yield\ (\%) = \frac{Weight\ of\ the\ Dried\ Extract}{Initial\ Weight\ of\ the\ Plant\ Material} \times 100$$

2.3 Determination of Extraction Yield

Extraction efficiency differed markedly among the solvent systems employed. The hydroethanolic solvent produced the highest extractive yield (21.11%), followed by the aqueous extract (15.14%) and the ethanolic extract (11.30%). The improved yield obtained with the hydroethanolic system indicates its greater capacity to extract a broad spectrum of phytoconstituents with varying polarities. This enhanced recovery is consistent with the well-established ability of hydroethanolic solvent mixtures to solubilize both polar and moderately polar bioactive compounds more effectively than single-solvent systems.

2.4 Elemental Analysis by ICP–MS

The elemental composition of the *M. paradisiaca* spathe extracts, with particular emphasis on iron content, was determined using Inductively Coupled Plasma–Mass Spectrometry (ICP–MS). Prior to analysis, each extract underwent acid digestion following a validated protocol for trace elemental analysis to ensure complete mineralization of the samples. Elemental quantification was performed using external calibration with certified multi-element standard solutions.

Iron was selected as the primary analytical marker because of its essential role in erythropoiesis and its direct relevance to iron deficiency-associated haematological disorders. Comparative ICP–MS analysis demonstrated significant variations in iron content among the different solvent extracts. The hydroethanolic extract contained the highest iron concentration, followed by the aqueous and ethanolic extracts, indicating that the hydroethanolic solvent system provided the most efficient recovery of iron and other mineral constituents from the banana spathe.

2.5 Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The phytochemical composition of the aqueous (AqE), ethanolic (EtE), and hydroethanolic (HAE-MP) extracts of *M. paradisiaca* spathe was characterized using Gas Chromatography–Mass Spectrometry (GC–MS). Analyses were carried out on an Agilent GC–MS system fitted with an HP-5 capillary column. Briefly, 1 µL of each extract was injected under optimized chromatographic conditions. Helium served as the carrier gas at a constant flow rate, while the mass spectrometer was operated in electron ionization (EI) mode over a scan range of m/z 35–550.

Separation of volatile and semi-volatile constituents was achieved using a programmed oven temperature gradient to ensure efficient resolution of compounds with different physicochemical properties. Total ion chromatograms (TICs) were acquired and processed using Agilent MassHunter software. Tentative identification of individual constituents was accomplished by matching the obtained mass spectra with those available in the National Institute of Standards and Technology (NIST) mass spectral library. The relative abundance of each detected compound was estimated by peak area normalization, enabling comparative phytochemical profiling of the three extracts.

2.6 In Vitro Scratch Wound Healing Assay and Inflammatory Gene Expression Analysis

The wound-healing potential of the aqueous (AqE), ethanolic (EtE), and hydroethanolic (HAE-MP) extracts of *M. paradisiaca* spathe was assessed using an in vitro scratch wound assay in RAW 264.7 murine macrophages. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum under standard culture conditions (37 °C in a humidified atmosphere containing 5% CO_2). When cell monolayers reached approximately 85–90% confluence in 6-well culture plates, they were serum-starved for 6 h to minimize cell proliferation and ensure that subsequent wound closure primarily reflected cell migration rather than proliferative activity.

A uniform linear scratch was generated using a sterile P200 pipette tip and the detached cells were removed by washing with phosphate-buffered saline (PBS). The initial wound area (0 h) was immediately documented using an inverted phase-contrast microscope (Olympus, Japan). Cells were subsequently exposed to cisplatin (10 µg/mL) for 6 h followed by treatment with aqueous, ethanolic, or hydroethanolic extracts of *M. paradisiaca* (0.5 mg/mL) for 18 h under serum-free conditions. Representative images were acquired at 0, 24, and 48 h, and wound closure was assessed by monitoring cellular migration into the scratched area.

To investigate the anti-inflammatory effects of the extracts, RAW 264.7 cells were treated with cisplatin (10 µg/mL) for 12 h followed by incubation with the respective extracts (0.5 mg/mL) for 24 h. Total RNA was isolated and reverse transcribed into cDNA. Quantitative real-time PCR (RT-qPCR) was performed using SYBR Green chemistry to determine the expression of inflammation-related genes including interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), interleukin-10 (IL-10), and transforming growth factor-beta (TGF-β). Gene expression levels were normalized against GAPDH and calculated using the $2^{-}\Delta\Delta Ct$ method.

2.7 Acute Oral Toxicity Study

The acute oral toxicity of the hydroethanolic extract of *M. paradisiaca* spathe was evaluated in accordance with OECD Guideline 423 (Acute Toxic Class Method). Female Wistar rats (8–10 weeks old; 180–220 g) were acclimatized under standard laboratory conditions and maintained with free access to standard pellet diet and water. The study protocol was approved by the Institutional Animal Ethics Committee (Approval No. TAAB/ATOX/073).

A limit test was performed at a dose level of 2000 mg/kg body weight administered orally by gastric gavage. In the first step, three animals received the test extract, and following the absence of mortality, an additional three animals were treated at the same dose for confirmation. The extract was suspended in 0.5% (w/v) carboxymethyl cellulose (CMC) and administered orally at a dosing volume not exceeding 10 mL/kg body weight. Animals were fasted overnight before treatment and monitored continuously for the first 4 h following administration, with subsequent observations conducted daily for 14 days. Toxicological assessment included evaluation of general behavior, autonomic and neurological responses, clinical signs of toxicity, morbidity, mortality, food and water consumption, and changes in body weight throughout the observation period.

2.8 Repeated-Dose 28-Day Oral Toxicity Study

The repeated-dose oral toxicity of the hydroethanolic *M. paradisiaca* spathe extract (HAE-MP) was evaluated following the procedures outlined in OECD Guideline 407. Healthy Wistar rats (6–8 weeks old) of both sexes were procured from an accredited animal facility and acclimatized for seven days under standard laboratory conditions. Animals were housed at 20–24°C with 30–70% relative humidity under a 12 h light/12 h dark photoperiod and provided a standard laboratory diet and water ad libitum.

A total of 48 rats (24 males and 24 females) were randomly allocated into four experimental groups (n = 12 per group; 6 males and 6 females). Group I served as the vehicle control and received distilled water, whereas Groups II, III, and IV were administered HAE-MP orally by gavage at doses of 200, 400, and 800 mg/kg body weight, respectively, once daily for 28 consecutive days. Throughout the study, animals were monitored daily for mortality, clinical signs of toxicity, behavioural alterations, food consumption, and changes in body weight.

At the completion of the treatment period, blood samples were collected for haematological and serum biochemical analyses. The animals were then euthanized and subjected to a comprehensive gross necropsy. Major organs were excised, weighed to determine relative organ weights, and examined for treatment-related abnormalities before comparison across the experimental groups.

2.9 *In vivo* anti-anaemic activity

The anti-anaemic efficacy of the hydroethanolic *M. paradisiaca* spathe extract (HAE-MP) was investigated using a phenylhydrazine (PHZ)-induced haemolytic anaemia model in Wistar rats. Experimental anaemia was induced by intraperitoneal administration of PHZ (40 mg/kg body weight) once daily for two consecutive days. The induction of anaemia was confirmed by a marked reduction in haemoglobin concentration and erythrocyte count prior to treatment initiation.

Following confirmation of anaemia, the animals were randomly assigned to four experimental groups (n = 6 per group): a normal control group, a PHZ-induced anaemic control group, an HAE-MP treatment group (500 mg/kg body weight, p.o.), and a standard treatment group receiving ferrous ascorbate (equivalent to 9 mg Fe/kg body weight, p.o.). Oral administration of the test extract and standard drug was carried out once daily for 13 consecutive days (Days 3–15) following PHZ-induced anaemia.

Blood samples were collected on Days 0, 3, 7, 10, and 15 through the retro-orbital plexus under light anesthesia. Haematological parameters, including red blood cell (RBC) count, haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), were determined using an automated haematology analyzer. At the end of the treatment period, serum was separated for estimation of serum iron, ferritin, total iron-binding capacity (TIBC), and transferrin saturation using commercially available diagnostic kits according to the manufacturers' protocols.

To investigate the effect of HAE-MP on erythropoiesis and oxidative stress associated with PHZ-induced haemolysis, reticulocyte count, serum erythropoietin (EPO), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were also determined using standard biochemical methods. Data were expressed as mean ± SEM (n = 6) and analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using GraphPad Prism software. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1 Extraction Yield and Iron Content of *M. paradisiaca* Spathe Extracts

The extraction yield and elemental iron content of the three *M. paradisiaca* spathe extracts are presented in Table 1. Both parameters were markedly influenced by the extraction solvent. The hydroethanolic extract exhibited the highest extraction yield (21.11%), corresponding to approximately 1.4-fold and 1.9-fold greater recovery than the aqueous and ethanolic extracts, respectively. Consistent with these findings, ICP–MS analysis revealed that the hydroethanolic extract contained the highest iron concentration (883.18 mg/kg), followed by the aqueous (740.32 mg/kg) and ethanolic (695.72 mg/kg) extracts.

The enhanced extraction yield together with the greater iron content indicates that the hydroethanolic solvent system was more effective in recovering both mineral constituents and a wider range of phytochemicals than either water or ethanol alone. Hydroethanolic solvent mixtures are well recognized for their ability to extract compounds spanning a broad polarity range, thereby improving the recovery of nutritionally and pharmacologically relevant constituents. The elevated iron concentration observed in the hydroethanolic extract is particularly significant, given the essential role of iron in haemoglobin synthesis, erythropoiesis, and the management of iron deficiency-associated anaemia.

Table 1. Extraction yield and iron content of M. paradisiaca spathe extracts

Extract	Solvent System	Extraction Yield (%)	Iron Content (mg/kg)
AgE	Water	15.14	740.32
EtE	Ethanol	11.30	695.72
HyA	Ethanol:Water (50:50)	21.11	883.18

These findings demonstrate that solvent composition plays a critical role in determining extraction efficiency and elemental composition. Owing to its superior extractive yield and highest iron content, the hydroethanolic extract was selected as the lead extract for subsequent phytochemical characterization, biological evaluation, and safety studies.

3.2. GC–MS Characterization of *M. paradisiaca* Spathe Extracts

The phytochemical composition of the aqueous (AqE), ethanolic (EtE), and hydroethanolic (HAE-MP) extracts of *M. paradisiaca* spathe was investigated using gas chromatography–mass spectrometry (GC–MS). Comparative analysis of

the total ion chromatograms (TICs) demonstrated distinct chemical fingerprints for each extract, highlighting the pronounced influence of solvent composition on metabolite recovery and phytochemical diversity.

The aqueous extract exhibited a comparatively simple chromatographic pattern with relatively few low-intensity peaks distributed across the chromatographic run (Figure 1A). Most detectable compounds eluted during the early retention period, indicating the predominance of highly polar constituents. The limited number of resolved peaks suggests that water alone extracted a narrower range of phytochemicals.

In contrast, the ethanolic extract produced a more complex chromatographic profile, characterized by several well-defined peaks with higher signal intensities, particularly between approximately 8 and 16 min (Figure 1B). These features indicate efficient extraction of moderately polar and semi-volatile metabolites, reflecting the greater affinity of ethanol for a broader range of bioactive constituents.

Among all extracts, the hydroethanolic extract displayed the richest and most chemically diverse chromatographic profile (Figure 1C). Numerous well-resolved peaks spanning the entire chromatographic run were observed, indicating the presence of a wide variety of metabolites with differing physicochemical properties. The broader peak distribution and greater chromatographic complexity suggest that the hydroethanolic solvent system enhanced the extraction of both polar and moderately non-polar phytochemicals. This observation is consistent with previous reports demonstrating that aqueous-organic solvent mixtures provide superior extraction efficiency by expanding the solvent polarity range, thereby facilitating the recovery of a more comprehensive spectrum of plant secondary metabolites.

Table 2. Tentative identification of major phytochemical constituents in the hydroethanolic extract of *M. paradisiaca* spathe based on GC–MS analysis.

RT (min)	Tentative Compound	Molecular Formula	MW	Chemical Class	Potential Biological Relevance
3.134	Oxacyclotetradeca-4,11-diyne	C13H18O	190.28	Oxygenated cyclic hydrocarbon	Antioxidant and membrane-protective activity
3.705	Benzyl (1,2,3-thiadiazol-4-yl) carbamate	C10H9N3O2S	235.26	Thiadiazole derivative	Antioxidant and cytoprotective potential
7.958	1-Benzylbenzimidazole 3-oxide	C14H12N2O	224.26	Benzimidazole derivative	Free-radical scavenging and anti-inflammatory activity
8.596	Diazabicyclic oxazepine derivative	C14H20N2O4	280.32	Nitrogen-containing heterocycle	Cytoprotective activity
9.216	Diazabicyclo-heptene derivative	C10H16N2O2	196.25	Bicyclic heterocycle	Antioxidant potential
9.923	Diazabicyclo-heptene derivative (isomer)	C10H16N2O2	196.25	Bicyclic heterocycle	Bioactive nitrogenous metabolite
10.299	Diazabicyclo-heptene derivative	C10H16N2O2	196.25	Bicyclic heterocycle	Potential cellular protective activity
10.499	Methyl 4-methylcyclohexyl propylphosphonate	C11H23O3P	234.27	Organophosphonate derivative	Minor constituent
10.725	4-Diazoadamantanone	C10H12N2O	176.22	Diazoketone derivative	Antioxidant-related scaffold
11.637	3β-Acetoxy-16-isothiocyanatopregn-5-en-20-one	C24H35NO3S	417.60	Steroidal isothiocyanate	Anti-inflammatory and cytoprotective activity

The GC–MS findings were consistent with the extraction yield and elemental composition analyses. The hydroethanolic extract produced the highest extraction yield (21.11%), followed by the aqueous (15.14%) and ethanolic (11.30%) extracts. Likewise, ICP–MS analysis demonstrated that the hydroethanolic extract contained the greatest iron concentration (883.18 mg/kg), compared with the aqueous (740.32 mg/kg) and ethanolic (695.72 mg/kg) extracts. The concurrent improvement in extraction efficiency, iron recovery, and phytochemical diversity indicates that the hydroethanolic solvent system was the most effective for extracting biologically relevant constituents from *M. paradisiaca* spathe.

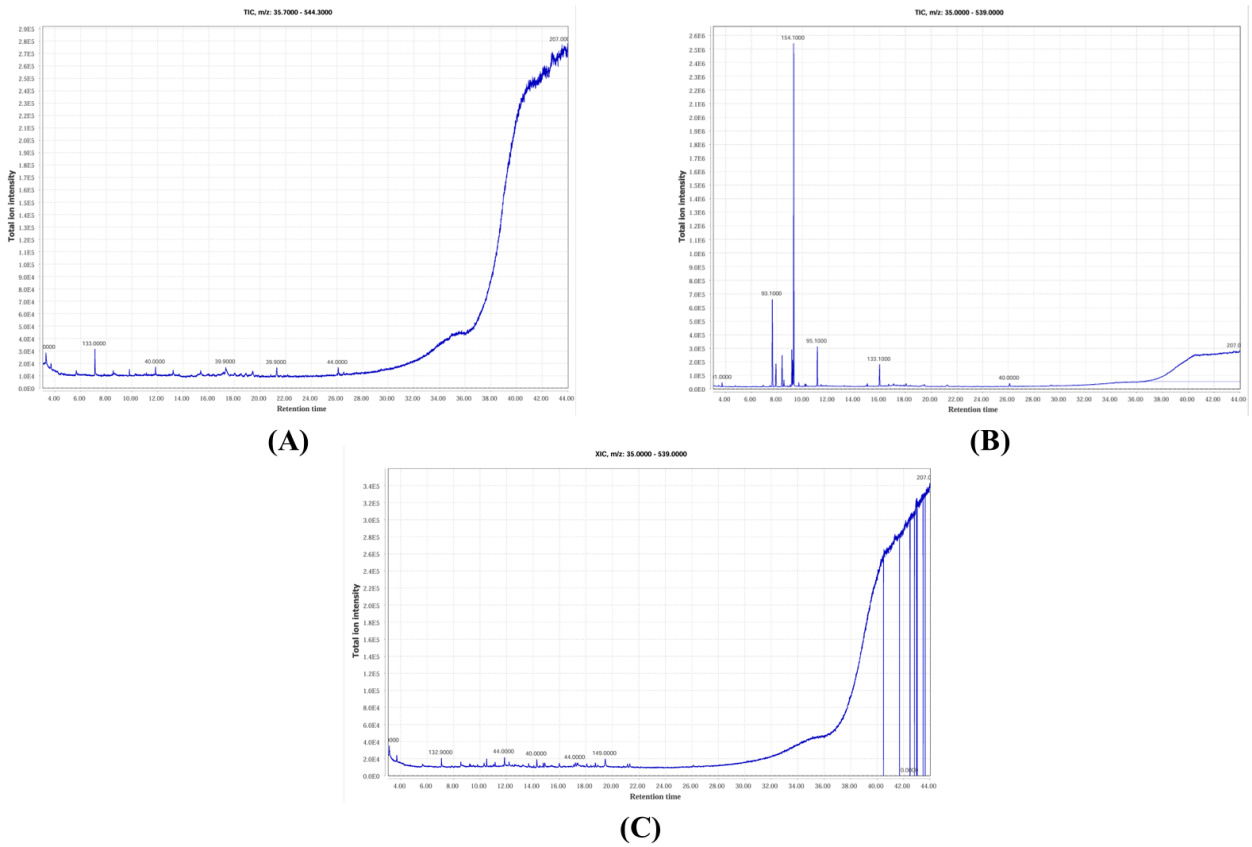

Figure 1. Total ion chromatograms (TICs) of (A) aqueous (AqE), (B) ethanolic (EtE), and (C) hydroethanolic (HyA) extracts of *M. paradisiaca* spathe obtained by GC–MS analysis.

Based on its superior extraction yield, elevated iron content, and comprehensive phytochemical profile, the hydroethanolic extract was selected for subsequent pharmacological and toxicological investigations. GC–MS analysis identified a chemically diverse array of metabolites, including oxygenated hydrocarbons, thiadiazole derivatives, benzimidazole analogues, diazabicyclic heterocycles, and steroidal compounds. The predominance of nitrogen-containing heterocyclic and steroid-like constituents suggests the presence of metabolites with potential biological activity. Previous studies have associated these classes of compounds with antioxidant, cytoprotective, and anti-inflammatory effects, which may partially explain the pharmacological responses observed in the present study. Collectively, the combination of a chemically diverse metabolite profile and high iron content indicates that the hydroethanolic extract possesses a multifaceted phytochemical composition capable of supporting erythropoiesis, reducing oxidative stress, and promoting haematological recovery.

3.3 In Vitro Wound Healing and Anti-Inflammatory Activity

The in vitro scratch wound assay demonstrated that the three *M. paradisiaca* spathe extracts differentially influenced the migratory capacity of RAW 264.7 macrophages following cisplatin-induced cellular injury. Untreated control cells exhibited progressive closure of the scratch area throughout the 48 h observation period, whereas cisplatin-treated cells showed markedly impaired migration and delayed wound closure. Treatment with the M. paradisiaca extracts significantly enhanced cellular migration compared with the injured control, although the magnitude of the response varied among the extracts.

The hydroethanolic extract produced the greatest wound-healing response, resulting in substantial restoration of the scratched monolayer within 48 h (Figure 2). In comparison, the aqueous extract exhibited moderate wound closure, while the ethanolic extract produced a comparatively weaker migratory response. The superior activity of the hydroethanolic extract is consistent with its higher extraction yield, greater iron content, and broader phytochemical diversity observed in the GC–MS analysis. These findings suggest that the hydroethanolic extract effectively alleviated cisplatin-induced impairment of macrophage migration and promoted cellular repair, supporting its potential wound-healing activity.

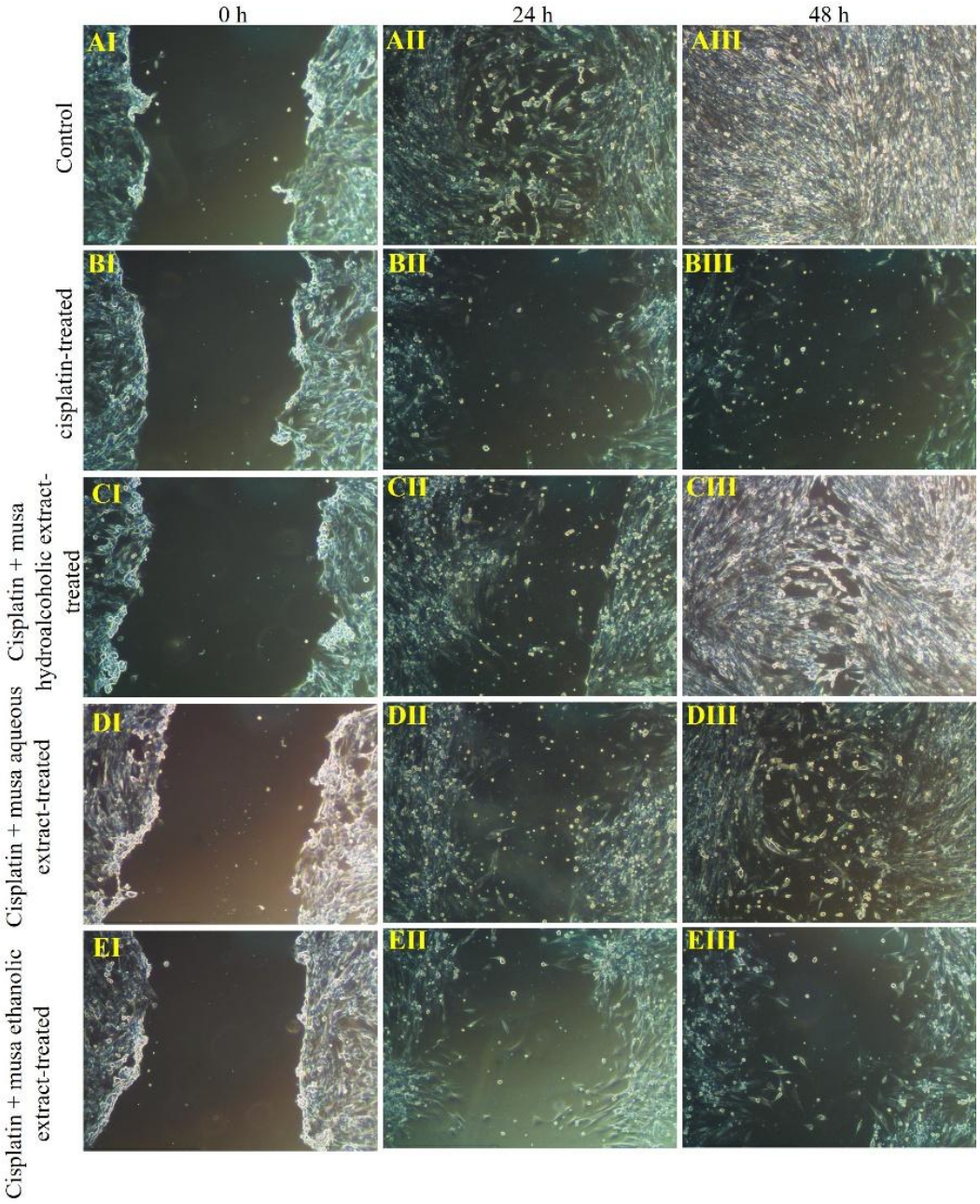

Figure 2. Representative phase-contrast micrographs illustrating the wound-healing response of RAW 264.7 macrophages in an in vitro scratch assay. A linear scratch was created using a sterile P20 pipette tip, and wound closure was monitored at 0, 24, and 48 h. Untreated control cells (AI–AIII) exhibited progressive migration and closure of the scratched area, whereas cisplatin-treated cells (10 µg/mL for 6 h; BI–BIII) showed markedly impaired wound closure. Subsequent treatment with the hydroethanolic extract of *M. paradisiaca* spathe (HAE-MP; 0.5 mg/mL for 18 h; CI–CIII) substantially restored cellular migration compared with the aqueous extract (AqE; 0.5 mg/mL for 18 h; DI–DIII) and ethanolic extract (EtE; 0.5 mg/mL for 18 h; EI–EIII). The hydroethanolic extract demonstrated the greatest enhancement of wound closure, indicating superior wound-healing potential among the tested extracts.

The anti-inflammatory activity of the *M. paradisiaca* spathe extracts was further evaluated by analyzing the expression of key inflammatory cytokines in RAW 264.7 macrophages. Exposure to cisplatin elicited a pronounced inflammatory response, evidenced by significant upregulation of the pro-inflammatory cytokines IL-6 and TNF-α, together with suppression of the anti-inflammatory cytokine IL-10. Treatment with the hydroethanolic extract (HAE-MP) markedly reduced the expression of IL-6 and TNF-α while restoring IL-10 expression toward basal levels, indicating effective modulation of the inflammatory response.

In comparison, the aqueous extract (AqE) produced a moderate anti-inflammatory effect, whereas the ethanolic extract (EtE) induced only limited changes in cytokine expression. The expression of TGF-β remained largely unchanged across all experimental groups, suggesting that the observed anti-inflammatory effects were primarily mediated through modulation of the IL-6/TNF-α/IL-10 signalling axis rather than alterations in TGF-β expression. Overall, these findings demonstrate that the hydroethanolic extract exhibited the strongest anti-inflammatory activity and effectively mitigated

cisplatin-induced inflammatory signalling in macrophages, supporting its potential role in promoting tissue repair and wound healing.

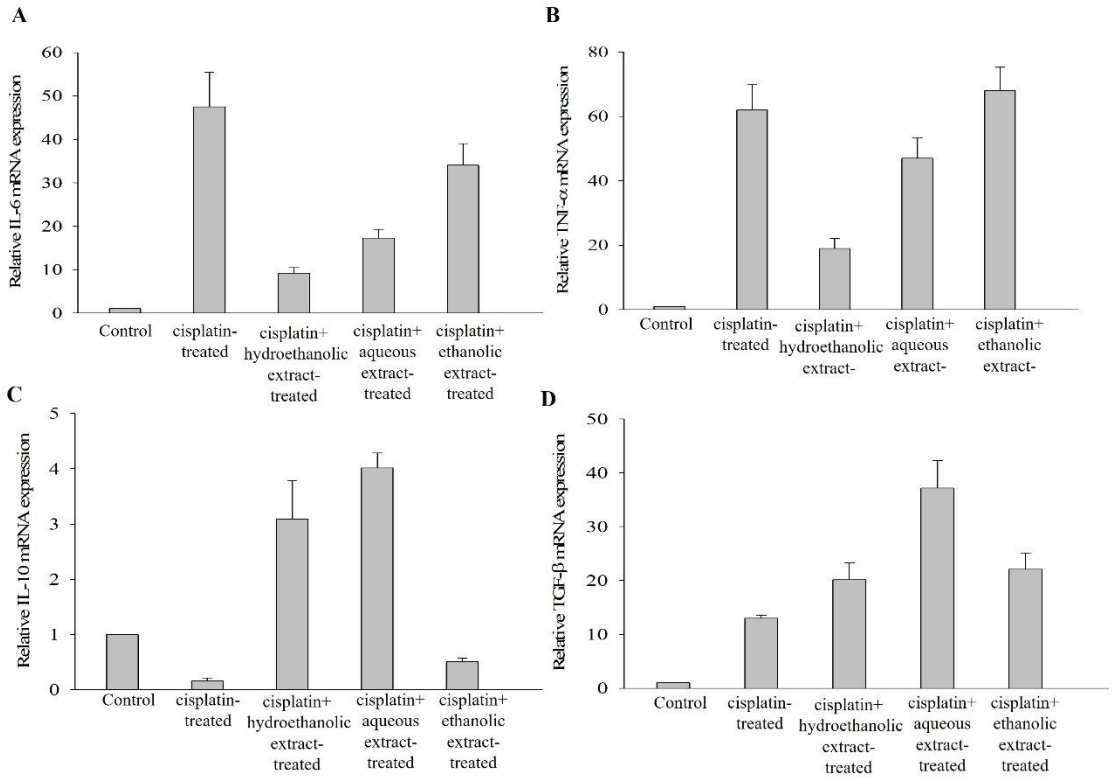

Figure 3. qRT-PCR analysis of inflammation-related gene expression in RAW 264.7 macrophages. Relative mRNA expression levels of pro- and anti-inflammatory cytokines (IL-6, TNF-α, IL-10, and TGF-β) were quantified by SYBR Green-based RT-qPCR following cisplatin exposure and subsequent treatment with hydroethanolic, aqueous and ethanolic extracts of *M. paradisiaca*. Gene expression was normalized to GAPDH and calculated using the 2−ΔΔCt method. Data are presented as mean ± SEM from three independent biological replicates.

The enhanced wound closure observed in hydroethanolic extract-treated cells may therefore be attributed, at least in part, to suppression of pro-inflammatory cytokines and restoration of anti-inflammatory signalling pathways. These results support the biological relevance of the hydroethanolic extract and justify its selection for subsequent *in vivo* safety and anti-anaemic evaluations.

3.4 Acute Oral Toxicity Assessment

Acute oral administration of the hydroethanolic extract of *M. paradisiaca* spathe at a limit dose of 2000 mg/kg produced no mortality during the 14-day observation period. All six treated animals survived throughout the study, indicating that the median lethal dose (LD_{50}) exceeded 2000 mg/kg body weight. According to OECD Guideline 423, the extract may therefore be classified as Category 5 or unclassified under the Globally Harmonized System (GHS) for acute oral toxicity. No treatment-related clinical signs of toxicity were observed following extract administration. Animals exhibited normal posture, locomotor activity, grooming behaviour, responsiveness to handling, respiratory pattern, food consumption, and water intake throughout the study. Furthermore, no tremors, convulsions, salivation, diarrhoea, piloerection, lacrimation, lethargy, or behavioural abnormalities were detected during the observation period. Functional observation battery assessment demonstrated normal neurological and autonomic responses at all evaluated time points.

Table 3. Mortality and acute toxicity classification of hydroethanolic extract of *M. paradisiaca* spathe

Dose (mg/kg)	Number of Animals	Mortality	LD_{50} Classification
2000	6	0/6	LD_{50} > 2000 mg/kg

Table 4. Body weight changes during OECD 423 acute oral toxicity study

Group	Day 0 (g)	Day 7 (g)	Day 14 (g)	Weight Gain (%)
Control	198.33 ± 5.99	205.67 ± 5.57	214.33 ± 5.99	8.08 ± 1.58
Extract (2000 mg/kg)	199.16 ± 7.10	207.50 ± 7.50	216.80 ± 7.30	8.88 ± 0.38
N=6				

Body weight increased progressively throughout the 14-day observation period in both the control and HAE-MP-treated groups. Animals receiving the hydroethanolic extract exhibited a significant increase in body weight from 199.16 ± 7.10 g on Day 0 to 216.80 ± 7.30 g on Day 14, corresponding to a mean weight gain of 8.88 ± 0.38%, which was comparable

to that observed in the control group. No treatment-related reduction in body weight or impairment of normal growth was detected, indicating that administration of the extract did not adversely affect the general health or physiological status of the animals.

The absence of mortality, clinical signs of toxicity, and treatment-related changes in body weight demonstrates that the hydroethanolic extract of *M. paradisiaca* spathe possesses a favourable acute oral safety profile. These findings suggest that HAE-MP is well tolerated following single-dose oral administration and may be considered practically non-toxic under the conditions of the present study.

3.4 Repeated-Dose 28-Day Oral Toxicity Study

Clinical Observations

No mortality or treatment-related adverse effects were observed throughout the 28-day repeated-dose oral toxicity study in any of the animals administered the hydroethanolic extract of *M. paradisiaca* spathe (HAE-MP). All treated rats remained clinically healthy and exhibited normal grooming behaviour, posture, locomotor activity, respiratory function, and responsiveness throughout the study period. Furthermore, no signs of toxicity, including tremors, convulsions, excessive salivation, diarrhoea, piloerection, lethargy, or neurological abnormalities, were observed at any of the tested dose levels. These findings demonstrate that repeated oral administration of HAE-MP was well tolerated and did not produce observable systemic or behavioural toxicity under the experimental conditions.

Body Weight Changes

Body weight increased progressively in both male and female rats throughout the 28-day treatment period (Table 5). In male animals, body weight gain in the 200 and 400 mg/kg HAE-MP treatment groups was comparable to that of the control group. Although rats receiving the highest dose (800 mg/kg) exhibited a marginally lower weight gain by Day 28, this difference was not associated with any clinical signs of toxicity or evidence of impaired health. Similarly, female rats across all treatment groups showed normal growth trajectories, with body weight gains comparable to or slightly exceeding those of the control group. The absence of treatment-related reductions in body weight or growth impairment indicates that repeated oral administration of HAE-MP did not adversely affect normal physiological development or nutritional status. These findings further support the favourable tolerability and safety profile of the hydroethanolic extract during repeated-dose exposure.

Table 5. Body weight changes following repeated oral administration of HAE-MP

Group	Dose (mg/kg)	Male Day 0 (g)	Male Day 28 (g)	Female Day 0 (g)	Female Day 28 (g)
Control	0	123.13 ± 1.48	140.21 ± 2.02	118.97 ± 1.40	136.64 ± 2.64
Low Dose	200	122.04 ± 1.50	138.46 ± 2.71	120.98 ± 1.05	138.74 ± 4.55
Mid Dose	400	122.89 ± 1.49	142.95 ± 1.99	120.44 ± 0.86	139.96 ± 2.64
High Dose	800	121.58 ± 1.27	132.82 ± 2.02	120.34 ± 2.03	141.07 ± 3.17
N=6 in each group					

Food Consumption

Food consumption remained normal throughout the 28-day treatment period in both male and female rats (Table 6). No treatment-related differences in feed intake were observed among the experimental groups. Male rats receiving the highest dose of HAE-MP (800 mg/kg) exhibited a slight reduction in food consumption during the later stages of the study; however, the decrease was minimal and was not accompanied by changes in body weight, clinical condition, or other indicators of systemic toxicity. Similarly, female rats maintained food intake comparable to that of the control group throughout the study. These findings indicate that repeated oral administration of HAE-MP did not adversely influence appetite, feeding behaviour, or overall nutritional status, further supporting the favourable safety and tolerability of the extract following repeated-dose exposure.

Table 6. Mean food consumption (g/animal/day) at Day 28

Group	Dose (mg/kg)	Male	Female
Control	0	8.58	7.39
Low Dose	200	8.15	7.73
Mid Dose	400	8.47	7.60
High Dose	800	7.94	7.35
N=6 in each group			

Haematological Findings

Repeated oral administration of the hydroethanolic *M. paradisiaca* spathe extract (HAE-MP) did not produce any treatment-related alterations in haematological parameters, and all measured values remained within normal physiological ranges throughout the study (Table 5). Haemoglobin concentration, erythrocyte count, haematocrit, total leukocyte count, platelet count, and erythrocyte indices showed no evidence of haematological toxicity at any of the tested dose levels.

A dose-dependent trend toward increased haemoglobin concentration, erythrocyte count, haematocrit, and platelet count was observed in both male and female rats receiving HAE-MP at 400 and 800 mg/kg. In male rats, administration of 800 mg/kg resulted in a haemoglobin concentration of 15.55 ± 0.65 g/dL and an erythrocyte count of 8.09 ± 0.31 × 10^6/mm^3, compared with 14.74 ± 1.71 g/dL and 6.75 ± 0.63 × 10^6/mm^3, respectively, in the control group. Likewise, female rats

treated with 800 mg/kg exhibited higher haemoglobin (15.82 ± 0.46 g/dL) and erythrocyte counts (7.57 ± 0.42 × 10^6/mm^3) than the corresponding controls. No evidence of anaemia, leukopenia, leucocytosis, thrombocytopenia, or other hematological abnormalities was detected in any treatment group. Although the observed increases in erythrocyte-related parameters remained within physiological limits, they may reflect a beneficial effect of HAE-MP on hematopoietic function, potentially attributable to its high iron content and diverse phytochemical composition. However, this association should be interpreted cautiously and warrants confirmation through dedicated mechanistic studies.

Serum Biochemical Parameters

Serum biochemical evaluation revealed no evidence of treatment-related hepatic or renal toxicity following repeated oral administration of the hydroethanolic *M. paradisiaca* spathe extract (HAE-MP) (Table 6). Hepatic function markers, including serum glutamic-pyruvic transaminase (SGPT/ALT) and serum glutamic-oxaloacetic transaminase (SGOT/AST), remained within normal physiological limits in both male and female rats. Similarly, renal function biomarkers, including blood urea nitrogen (BUN) and serum creatinine, showed no biologically significant changes across the treatment groups. Total serum protein concentrations remained comparable to those of the control group, indicating preservation of hepatic protein synthetic capacity throughout the treatment period. In addition, blood glucose levels remained within normal physiological ranges, suggesting that repeated administration of HAE-MP did not adversely affect glucose homeostasis. Overall, these biochemical findings demonstrate that repeated exposure to HAE-MP was well tolerated and did not produce detectable hepatic or renal dysfunction, further supporting its favourable safety profile following 28 days of oral administration.

Organ Weight Assessment

Absolute organ weight measurements revealed no biologically significant treatment-related changes in the liver, kidneys, or heart of treated animals compared with controls (Table 7). Organ weights remained proportional to terminal body weights and did not indicate organ enlargement, atrophy, or target-organ toxicity.

The absence of meaningful alterations in organ weights further supports the systemic safety of the extract following repeated oral administration.

Gross Necropsy Findings

Gross pathological examination performed at study termination did not reveal any treatment-related abnormalities. Major organs, including the liver, kidneys, heart, lungs, spleen, stomach, and gastrointestinal tract, appeared normal in size, colour, consistency, and anatomical architecture. No evidence of congestion, haemorrhage, ulceration, necrosis, or abnormal tissue growth was observed in any animal.

Histopathological Findings

Histopathological examination of the heart, kidneys, liver, lungs, and stomach revealed no treatment-related microscopic lesions following repeated oral administration of the hydroethanolic *M. paradisiaca* spathe extract (HAE-MP) at 800 mg/kg body weight for 28 consecutive days. Tissue architecture in all examined organs was comparable to that of the control group, with no evidence of treatment-induced structural abnormalities (Figure 4). Cardiac sections exhibited normal myocardial architecture with intact cardiac muscle fibres, endocardium, and pericardium, without evidence of degeneration, necrosis, inflammatory cell infiltration, fibrosis, or vascular congestion. Renal sections showed well-preserved glomeruli, renal tubules, interstitial tissue, and blood vessels, with no histological features suggestive of nephrotoxicity. Similarly, liver sections demonstrated normal hepatic cords, portal triads, and sinusoidal architecture, with no signs of hepatocellular degeneration, steatosis, necrosis, fibrosis, or inflammatory infiltration. Histological examination of the lungs revealed intact alveolar structures, bronchioles, and interstitial spaces, with no evidence of oedema, haemorrhage, fibrosis, granulomatous lesions, or other pathological changes. Gastric tissues also maintained normal mucosal, submucosal, muscular, and serosal architecture, without ulceration, erosion, inflammatory changes, or epithelial hyperplasia. The absence of treatment-related histopathological alterations across all examined organs is consistent with the haematological, serum biochemical, organ weight, and gross necropsy findings. These results demonstrate that repeated oral administration of HAE-MP for 28 days did not induce target-organ toxicity, further supporting its favourable safety profile under the conditions of the present study.

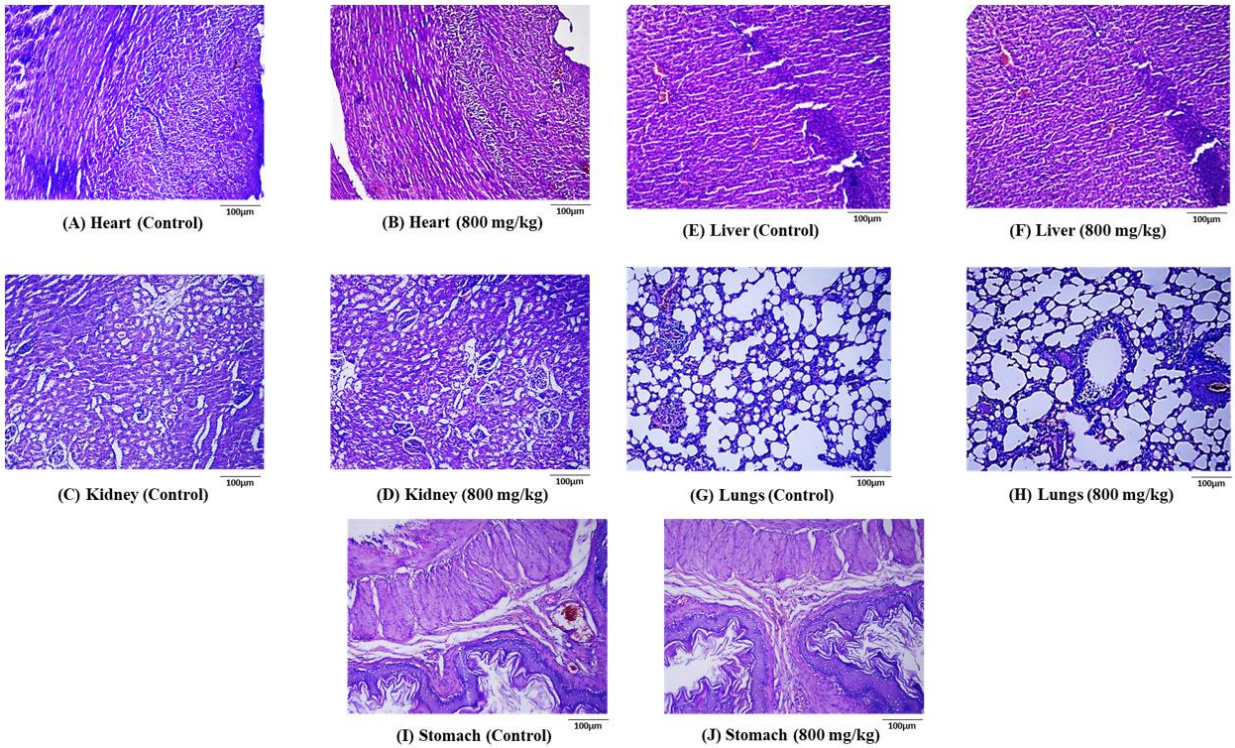

Figure 4. Representative haematoxylin and eosin (H&E)-stained sections of major organs following repeated oral administration of the hydroethanolic *M. paradisiaca* spathe extract (HAE-MP; 800 mg/kg) for 28 consecutive days. (A) Heart, control; (B) Heart, HAE-MP; (C) Kidney, control; (D) Kidney, HAE-MP; (E) Liver, control; (F) Liver, HAE-MP; (G) Lung, control; (H) Lung, HAE-MP; (I) Stomach, control; and (J) Stomach, HAE-MP. Histological examination revealed preserved tissue architecture in all examined organs, with no evidence of treatment-related degeneration, necrosis, inflammatory infiltration, fibrosis, vascular congestion, or other pathological alterations. These observations are consistent with the haematological, serum biochemical, organ weight, and gross necropsy findings, confirming the absence of target-organ toxicity following repeated oral administration of HAE-MP. Scale bar = 100 µm.

3.6 Anti-anaemic activity of HAE-MP in phenylhydrazine-induced anemic rats

Phenylhydrazine administration successfully induced haemolytic anaemia, as evidenced by marked reductions in erythrocyte count, haemoglobin concentration, and haematocrit compared with the normal control group (Table 7). Oral administration of HAE-MP (500 mg/kg) for 13 consecutive days significantly improved all haematological parameters in comparison with the untreated anaemic group. RBC count progressively increased from 3.28 ± 0.24 × 10^6/µL on Day 3 to 7.46 ± 0.32 × 10^6/µL on Day 15, whereas the anaemic control group exhibited only partial spontaneous recovery (5.18 ± 0.28 × 10^6/µL). Similarly, haemoglobin concentration increased to 13.82 ± 0.31 g/dL in the HAE-MP-treated group compared with 11.38 ± 0.28 g/dL in the anaemic control group, while haematocrit recovered to 43.06 ± 1.15%, approaching the normal physiological range. Ferrous ascorbate treatment produced the greatest restoration of haematological indices throughout the study (Table 7).

Table 7. Effect of HAE-MP on haematological parameters in phenylhydrazine-induced anaemic rats

Parameter	Day	Normal Control	Anaemic Control	HAE-MP (500 mg/kg)	Ferrous Ascorbate
RBC (×10^6/µL)	0	8.42 ± 0.31	8.55 ± 0.28	8.47 ± 0.26	8.61 ± 0.30
	3	8.31 ± 0.27	3.12 ± 0.21***	3.28 ± 0.24	3.19 ± 0.18
	7	8.28 ± 0.35	3.86 ± 0.26***	5.62 ± 0.31**	6.05 ± 0.27***
	10	8.36 ± 0.29	4.31 ± 0.33***	6.47 ± 0.35***	7.03 ± 0.29***
	15	8.41 ± 0.24	5.18 ± 0.28***	7.46 ± 0.32***	7.95 ± 0.26***
Hemoglobin (g/dL)	0	14.32 ± 0.42	14.56 ± 0.36	14.48 ± 0.38	14.71 ± 0.40
	3	14.18 ± 0.37	8.72 ± 0.41***	8.95 ± 0.39	8.84 ± 0.35
	7	14.24 ± 0.33	9.25 ± 0.36***	11.42 ± 0.41**	11.98 ± 0.34***
	10	14.37 ± 0.29	10.11 ± 0.32***	12.56 ± 0.37***	13.28 ± 0.29***
	15	14.52 ± 0.26	11.38 ± 0.28***	13.82 ± 0.31***	14.42 ± 0.27***
Hematocrit (%)	0	43.56 ± 1.85	44.21 ± 1.64	44.85 ± 1.71	45.18 ± 1.57
	3	43.12 ± 1.61	24.74 ± 1.12***	25.31 ± 1.24	24.88 ± 1.09
	7	42.86 ± 1.53	27.56 ± 1.27***	37.68 ± 1.48***	39.42 ± 1.36***
	10	43.28 ± 1.42	30.84 ± 1.31***	40.85 ± 1.33***	42.64 ± 1.24***
	15	43.81 ± 1.28	34.92 ± 1.26***	43.06 ± 1.15***	45.32 ± 1.08***
N=6 in each group					

Values are expressed as mean ± SEM (n = 6). **p < 0.01, ***p < 0.001 versus normal control (Day-matched comparison). Significant improvements in iron metabolism were also observed following HAE-MP treatment (Table 8). Serum iron concentration increased by approximately 80% compared with the anaemic control group, accompanied by restoration of serum ferritin and transferrin saturation. Conversely, the elevated total iron-binding capacity observed following phenylhydrazine administration was significantly reduced after treatment. At Day 15, serum iron, ferritin, TIBC, and transferrin saturation in the HAE-MP-treated animals were 148.5 ± 6.4 µg/dL, 72.4 ± 4.1 ng/mL, 336.8 ± 10.8 µg/dL, and 32.7 ± 1.8%, respectively, whereas ferrous ascorbate produced values closer to those of the normal control group (Table X).

Table 8. Effect of HAE-MP on iron profile and erythropoietic biomarkers in phenylhydrazine-induced anaemic rats

Parameter	Normal Control	Anaemic Control	HAE-MP (500 mg/kg)	Ferrous Ascorbate
Serum iron (µg/dL)	172.4 ± 8.2	82.6 ± 5.8***	148.5 ± 6.4***	164.2 ± 7.1***
Ferritin (ng/mL)	86.7 ± 4.5	41.3 ± 3.2***	72.4 ± 4.1***	81.8 ± 3.7***
TIBC (µg/dL)	305.8 ± 12.6	432.7 ± 15.4***	336.8 ± 10.8***	318.4 ± 11.6***
Transferrin saturation (%)	38.6 ± 2.1	18.4 ± 1.4***	32.7 ± 1.8***	36.9 ± 2.0***
Reticulocyte count (%)	2.12 ± 0.21	6.84 ± 0.43***	3.18 ± 0.29###	2.56 ± 0.25###
Serum EPO (mIU/mL)	18.6 ± 1.4	58.4 ± 3.8***	27.5 ± 2.1###	22.8 ± 1.8###
N=6 in each group				

Values are expressed as mean ± SEM (n = 6). ***p < 0.001 versus normal control; ###p < 0.001 versus anaemic control. Evaluation of erythropoietic biomarkers further demonstrated recovery from anaemia following extract administration (Table 9). Phenylhydrazine-induced anaemia markedly increased reticulocyte count (6.84 ± 0.43%) and serum erythropoietin concentration (58.4 ± 3.8 mIU/mL). Treatment with HAE-MP significantly normalized these parameters, reducing reticulocyte count to 3.18 ± 0.29% and erythropoietin concentration to 27.5 ± 2.1 mIU/mL. Ferrous ascorbate showed a slightly greater normalization of both biomarkers.

HAE-MP also significantly attenuated oxidative stress associated with haemolytic anaemia (Table X). Phenylhydrazine administration markedly increased lipid peroxidation, with MDA levels rising to 5.76 ± 0.34 nmol/mg protein, while endogenous antioxidant defences were substantially depleted. Treatment with HAE-MP reduced MDA to 2.48 ± 0.19 nmol/mg protein and significantly restored SOD, CAT, and GSH levels to 10.7 ± 0.6 U/mg protein, 55.8 ± 2.9 U/mg protein, and 7.01 ± 0.36 µmol/g tissue, respectively. Although the standard ferrous ascorbate group consistently demonstrated slightly greater efficacy, HAE-MP produced significant improvements across all haematological, iron metabolism, erythropoietic, and oxidative stress parameters compared with the untreated anaemic control group ($p < 0.001$).

Table 9. Effect of HAE-MP on oxidative stress biomarkers in phenylhydrazine-induced anemic rats

Parameter	Normal Control	Anemic Control	HAE-MP (500 mg/kg)	Ferrous Ascorbate
MDA (nmol/mg protein)	1.84 ± 0.12	5.76 ± 0.34***	2.48 ± 0.19###	2.11 ± 0.16###
SOD (U/mg protein)	12.8 ± 0.8	5.1 ± 0.4***	10.7 ± 0.6###	11.9 ± 0.7###
CAT (U/mg protein)	65.4 ± 3.6	28.6 ± 2.4***	55.8 ± 2.9###	61.7 ± 3.1###
GSH (µmol/g tissue)	8.42 ± 0.48	3.12 ± 0.28***	7.01 ± 0.36###	7.86 ± 0.42###
N=6 in each group				

Values are expressed as mean ± SEM (n = 6). ***p < 0.001 versus normal control; ###p < 0.001 versus anaemic control.

4. Discussion

The present study demonstrates that the hydroethanolic extract of *M. paradisiaca* spathe (HAE-MP) possesses a favourable combination of phytochemical richness, elevated iron content, biological activity, and toxicological safety, underscoring the therapeutic potential of this underutilized agricultural by-product. Although various parts of Musa species have been extensively investigated for their nutritional and medicinal applications, the spathe has received relatively limited scientific attention despite being a potential source of phenolic compounds, flavonoids, dietary fibre, essential minerals, and other bioactive constituents. Comprehensive characterization of this botanical resource is therefore essential to support its future development as a scientifically validated nutraceutical or phytopharmaceutical ingredient.

A major finding of the present investigation was the superior extraction performance of the hydroethanolic solvent system compared with aqueous or ethanolic extraction alone. Extraction solvent composition plays a critical role in determining the recovery of plant secondary metabolites, and hydroethanolic mixtures are widely recognized for their ability to extract compounds spanning a broad polarity range. Consistent with this principle, HAE-MP exhibited the highest extraction yield together with the greatest iron concentration among the evaluated extracts. These findings agree with those reported by (Jaleel et al. 2024), who described banana floral tissues as rich sources of phenolics, flavonoids, vitamins, sterols, and essential minerals, with extraction

efficiency strongly influenced by solvent selection. Likewise, (Sindhuja Lakshmi et al. 2024) demonstrated that hydroethanolic extracts of banana blossom possess higher concentrations of phenolic and flavonoid compounds and exhibit superior antioxidant activity compared with extracts prepared using single-solvent systems.

GC–MS characterization further demonstrated that HAE-MP contained the most chemically diverse metabolite profile among the investigated extracts. The detection of oxygenated compounds, nitrogen-containing heterocyclic metabolites, and steroid-like constituents suggests that the hydroethanolic solvent system effectively recovered structurally diverse phytochemicals with potential biological relevance. Although compound identification by GC–MS remains tentative, several of these chemical classes have previously been associated with antioxidant, anti-inflammatory, and cytoprotective activities. It is therefore unlikely that the pharmacological effects observed in the present study arise from a single bioactive molecule. Instead, they are more plausibly explained by synergistic interactions among multiple phytochemical constituents, a characteristic feature of botanical medicines. Similar conclusions have been drawn by (Ranjha et al. 2022) and Mathew and Negi 2017), who highlighted the broad spectrum of secondary metabolites present in Musa species and their complementary roles in modulating oxidative stress, inflammation, glucose metabolism, and tissue regeneration through multiple molecular mechanisms.

Another important finding of the present study was the high iron concentration detected in HAE-MP by ICP–MS analysis. Iron is an essential micronutrient required for heme biosynthesis, erythropoiesis, oxygen transport, and numerous enzymatic processes involved in cellular metabolism and energy production. The elevated iron content of HAE-MP therefore provides a plausible nutritional basis for the hematinic activity subsequently observed in the phenylhydrazine-induced anaemia model. Nevertheless, the therapeutic effects of the extract are unlikely to be attributable solely to its iron content.

Growing evidence indicates that plant-derived polyphenols, flavonoids, and other antioxidant phytochemicals play complementary roles in supporting haematological function by mitigating oxidative stress, preserving erythrocyte membrane integrity, and enhancing endogenous antioxidant defence mechanisms. Through these actions, such bioactive constituents may improve the efficiency of erythropoiesis and promote the effective utilization of available iron during haemoglobin synthesis. Consequently, the combined presence of bioavailable iron and antioxidant phytochemicals in HAE-MP may provide a multifaceted therapeutic advantage over conventional iron supplementation alone. This integrated mechanism could contribute to improved haematological recovery while simultaneously protecting erythrocytes from oxidative damage, although further mechanistic studies are required to confirm these interactions.

The in vitro scratch wound assay and cytokine expression analyses further demonstrated the biological relevance of the phytochemical composition of HAE-MP. Compared with the aqueous and ethanolic extracts, HAE-MP produced the greatest enhancement of macrophage migration following cisplatin-induced injury and significantly downregulated the pro-inflammatory cytokines IL-6 and TNF-α while restoring the expression of the anti-inflammatory cytokine IL-10. These findings indicate that HAE-MP effectively modulates the inflammatory microenvironment associated with tissue injury. Controlled resolution of inflammation is a critical prerequisite for successful wound healing, as persistent production of pro-inflammatory mediators can impair fibroblast proliferation, collagen synthesis, angiogenesis, and tissue remodelling. The present observations are consistent with previous studies demonstrating the wound-healing and anti-inflammatory properties of *M. paradisiaca*. (Cheng et al. 2024) reported that Musa extracts accelerated wound closure, enhanced collagen deposition, and promoted tissue regeneration in diabetic wound models. Similarly, (Amutha et al. 2014) demonstrated that *M. paradisiaca* stem extract significantly improved wound contraction and epithelialization, attributing these effects to its antioxidant-rich phytochemical composition. These findings suggest that the wound-healing activity of HAE-MP is mediated through coordinated antioxidant and immunomodulatory mechanisms rather than direct stimulation of cell proliferation alone. The extraction yield, elemental composition, phytochemical profile, and in vitro biological activity indicate that hydroethanolic extraction is an effective approach for recovering iron-rich and phytochemically diverse fractions from *M. paradisiaca* spathe. The integration of ICP–MS, GC–MS, and cell-based functional assays provides complementary evidence supporting HAE-MP as a promising botanical extract with both nutritional and pharmacological value, thereby providing a strong rationale for its subsequent toxicological and *in vivo* anti-anaemic evaluation.

Establishing a comprehensive toxicological profile is a critical requirement for the development of botanical products intended for long-term therapeutic use. Although *M. paradisiaca* has been widely consumed as a food source and used in traditional medicine, the safety of its spathe has not been extensively investigated. To address this knowledge gap, the present study evaluated the safety of the hydroethanolic *M. paradisiaca* spathe extract (HAE-MP) using both acute oral toxicity (OECD Guideline 423) and repeated-dose 28-day oral toxicity (OECD Guideline 407) protocols before investigating its pharmacological efficacy. In the acute toxicity study, administration of HAE-MP at a limit dose of 2000 mg/kg produced no mortality, treatment-related clinical signs, behavioural abnormalities, or gross pathological changes, indicating a wide margin of acute safety. In addition, the progressive increase in body weight observed throughout the 14-day observation period suggests that the extract did not adversely affect normal growth, metabolic function, or general health, parameters that are widely recognized as sensitive indicators of systemic tolerability. The findings of the repeated-dose toxicity study further confirmed the favourable safety profile of HAE-MP. Daily oral administration for 28 consecutive days produced no evidence of cumulative or dose-dependent toxicity. Food consumption and body weight gain remained within normal physiological ranges, while

haematological and serum biochemical parameters showed no treatment-related abnormalities. Similarly, organ weights and gross necropsy findings were comparable to those of the control group. Histopathological examination of the heart, liver, kidneys, lungs, and stomach revealed preserved tissue architecture with no evidence of inflammatory infiltration, cellular degeneration, necrosis, fibrosis, vascular congestion, or other treatment-related lesions. Collectively, these findings demonstrate that HAE-MP was well tolerated and did not induce target-organ toxicity, even at the highest tested dose of 800 mg/kg, supporting its suitability for further preclinical pharmacological development.

The toxicological findings of the present study are consistent with previous reports on the safety of *M. paradisiaca* extracts. (Ugbogu et al. 2018) demonstrated that repeated administration of fermented *M. paradisiaca* extract produced no significant alterations in biochemical, haematological, or histopathological parameters, concluding that the extract was well tolerated at doses up to 800 mg/kg in experimental animals. Similarly, (Bera et al. 2013) reported that petroleum ether, ethyl acetate, and methanolic leaf extracts of *M. paradisiaca* did not induce mortality or clinically relevant biochemical changes following repeated administration. Although these studies evaluated different plant parts and extraction methods, their findings collectively support the favourable safety profile of *M. paradisiaca* and are in agreement with the absence of treatment-related toxicity observed for the hydroethanolic spathe extract in the present investigation. An additional observation of particular interest was the maintenance of normal haematological homeostasis following prolonged administration of HAE-MP. Rather than inducing haematological suppression, treatment with 400 and 800 mg/kg produced a modest dose-dependent increase in haemoglobin concentration, erythrocyte count, haematocrit, and platelet count in both male and female rats. Importantly, these changes remained within physiological reference ranges and were not accompanied by abnormalities in leukocyte counts, erythrocyte indices, or serum biochemical parameters, suggesting an adaptive physiological response rather than a toxicological effect. Given the high iron content identified by ICP–MS together with the chemically diverse phytochemical profile revealed by GC–MS, these findings may indicate nutritional support of normal erythropoiesis rather than direct pharmacological stimulation of haematopoiesis. Comparable improvements in haematological indices without evidence of systemic toxicity have also been reported for other iron-rich botanical preparations. The preservation of hepatic and renal function further reinforces the safety profile of HAE-MP. Liver function markers (ALT/SGPT and AST/SGOT), renal biomarkers (blood urea nitrogen and serum creatinine), total serum protein, and blood glucose remained within normal physiological limits throughout the treatment period, indicating that repeated exposure did not adversely affect hepatic metabolic activity or renal excretory function. These biochemical findings were fully corroborated by histopathological examination, which demonstrated preserved tissue architecture and an absence of treatment-related lesions in the heart, liver, kidneys, lungs, and stomach. The concordance between functional biomarkers and microscopic observations provides robust evidence supporting the systemic safety of HAE-MP. In accordance with OECD Guideline 407, the absence of treatment-related clinical signs, organ pathology, haematological abnormalities, biochemical disturbances, and histopathological lesions indicates that repeated oral administration of HAE-MP does not produce adverse systemic effects under the conditions of the present study. The favourable safety profile established in the present investigation has important implications for the future development of HAE-MP as a botanical therapeutic. Because interventions for chronic conditions such as iron deficiency-associated anaemia often require prolonged administration, a comprehensive demonstration of safety is essential alongside evidence of pharmacological efficacy. The absence of acute and repeated-dose toxicity, together with normal haematological, biochemical, and histopathological findings, indicates that HAE-MP is well tolerated following oral administration. The integration of OECD-compliant toxicological evaluation with detailed histopathological assessment therefore provides a robust preclinical safety foundation, supporting the continued investigation of *M. paradisiaca* spathe as a safe, evidence-based nutraceutical or phytopharmaceutical candidate. Following confirmation of its safety, the anti-anaemic potential of HAE-MP was evaluated using the phenylhydrazine (PHZ)-induced haemolytic anaemia model, one of the most widely accepted experimental models for assessing hematinic agents. PHZ induces oxidative damage to erythrocytes through the generation of reactive oxygen species (ROS), resulting in haemoglobin oxidation, lipid peroxidation of erythrocyte membranes, and premature destruction of circulating red blood cells. These pathological events lead to reduced haemoglobin concentration, decreased erythrocyte count, compensatory erythropoietic stress, and disruption of systemic iron homeostasis. Owing to its reproducibility and close resemblance to oxidative haemolytic anaemia, this model provides an appropriate platform for evaluating compounds with antioxidant, cytoprotective, and hematopoietic properties (Moreau et al., 2012; Shwetha et al., 2019).

In the present study, phenylhydrazine (PHZ) administration successfully induced haemolytic anaemia, as evidenced by significant reductions in erythrocyte count, haemoglobin concentration, haematocrit, serum iron, ferritin, and transferrin saturation, together with marked increases in total iron-binding capacity (TIBC), reticulocyte count, serum erythropoietin, and lipid peroxidation. Oral treatment with the hydroethanolic *M. paradisiaca* spathe extract (HAE-MP) significantly ameliorated these pathological alterations, restoring most haematological and biochemical parameters toward normal physiological values. Although the standard treatment, ferrous ascorbate, produced a slightly greater therapeutic response, HAE-MP consistently improved all evaluated biomarkers, demonstrating meaningful hematinic efficacy rather than merely alleviating the clinical manifestations of anaemia.

A particularly important finding was the improvement in systemic iron homeostasis following HAE-MP administration. ICP–MS analysis confirmed that the

hydroethanolic extract contained substantially higher iron levels than the aqueous and ethanolic extracts, indicating that HAE-MP serves as a natural source of iron within a phytochemically rich matrix. Treatment with HAE-MP significantly restored serum iron and ferritin concentrations, normalized transferrin saturation, and reduced TIBC, collectively indicating improved iron availability for erythropoiesis. In contrast to conventional iron supplements, which primarily replenish iron stores, botanical preparations enriched with both iron and antioxidant phytochemicals may provide complementary benefits by enhancing iron utilization while simultaneously protecting erythrocytes from oxidative damage. Similar improvements in iron metabolism and haematological recovery have been reported in PHZ-induced anaemia models treated with antioxidant-rich botanical extracts, supporting the concept that restoration of iron homeostasis is a key contributor to the recovery of erythropoietic function (Ousaaid et al., 2022).

Improvement in erythrocytic indices following HAE-MP treatment was accompanied by normalization of key erythropoietic biomarkers. As expected, PHZ-induced haemolysis produced marked elevations in circulating erythropoietin (EPO) and reticulocyte counts as compensatory responses to accelerated erythrocyte destruction and tissue hypoxia. Treatment with HAE-MP significantly reduced both EPO and reticulocyte levels, indicating restoration of oxygen-carrying capacity and a reduction in erythropoietic stress. Similar observations have been reported by Moreau et al., who demonstrated that PHZ-induced oxidative haemolysis triggers pronounced reticulocytosis and extramedullary erythropoiesis as adaptive responses to red blood cell loss. Therefore, the normalization of EPO and reticulocyte counts following HAE-MP administration suggests improved erythrocyte survival and recovery of haematological homeostasis rather than merely stimulating compensatory erythropoiesis. The antioxidant properties of HAE-MP likely represent another important mechanism underlying its anti-anaemic activity. Oxidative stress is a major contributor to PHZ-induced haemolytic anaemia, as excessive reactive oxygen species (ROS) promote lipid peroxidation of erythrocyte membranes, leading to membrane instability and premature haemolysis. Consistent with this mechanism, untreated anaemic rats exhibited significantly elevated malondialdehyde (MDA) levels together with depletion of endogenous antioxidant defences, including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). HAE-MP treatment significantly attenuated lipid peroxidation while restoring the activities of these endogenous antioxidant enzymes, demonstrating effective protection against oxidative injury. These findings are consistent with previous reports showing that antioxidant-rich botanical extracts enhance haematological recovery in PHZ-induced anaemia by preserving erythrocyte membrane integrity, reducing oxidative stress, and maintaining endogenous antioxidant defence systems (Prasad et al., 2018; Ousaaid et al., 2022).

The *in vivo* antioxidant effects of HAE-MP are consistent with its phytochemical profile characterized in the present study. GC–MS analysis revealed a chemically diverse extract containing oxygenated metabolites, nitrogen-containing heterocyclic compounds, and steroid-like constituents, several of which belong to chemical classes previously associated with antioxidant, anti-inflammatory, and cytoprotective activities. In combination with the elevated iron content identified by ICP–MS, these findings suggest that HAE-MP exerts its hematinic effects through complementary mechanisms involving both nutritional iron supplementation and protection of erythrocytes against oxidative damage. This multimodal mode of action is a recognized advantage of botanical preparations, which frequently derive their therapeutic efficacy from the synergistic interaction of multiple bioactive constituents rather than a single active compound. The present findings indicate that the anti-anaemic activity of HAE-MP is mediated through the coordinated regulation of iron homeostasis, erythropoiesis, and oxidative stress. Significant improvements in erythrocytic indices, iron metabolism, erythropoietic biomarkers, and endogenous antioxidant defences, together with the absence of treatment-related toxicity in both OECD Guideline 423 and OECD Guideline 407 studies, demonstrate that HAE-MP possesses a favourable balance of efficacy and safety. These results support the potential of *M. paradisiaca* spathe as a sustainable and scientifically validated botanical candidate for the management of iron deficiency-associated anaemia. Nevertheless, further investigations incorporating LC–MS-based metabolite characterization, pharmacokinetic studies of iron bioavailability, and mechanistic evaluation of iron regulatory pathways, particularly the hepcidin–ferroportin axis, will be important to fully elucidate the molecular basis of its hematinic activity and facilitate its future translational development.

5. Conclusion

The present study demonstrates that the hydroethanolic extract of *M. paradisiaca* spathe (HAE-MP) is a safe and biologically active botanical extract with promising therapeutic potential. Compared with aqueous and ethanolic extracts, HAE-MP exhibited the highest extraction yield, greatest iron content, and the most diverse phytochemical profile, which correlated with superior wound-healing, anti-inflammatory, and anti-anaemic activities. Acute and repeated-dose toxicity studies conducted according to OECD Guidelines 423 and 407 confirmed an excellent safety profile, with no evidence of treatment-related toxicity. In the phenylhydrazine-induced haemolytic anaemia model, HAE-MP significantly improved haematological indices, restored iron homeostasis, reduced oxidative stress, and normalized erythropoietic biomarkers. These therapeutic effects are likely mediated by the synergistic actions of its naturally occurring iron and diverse phytoconstituents, which collectively enhance iron utilization, antioxidant defence, and inflammatory regulation. Overall, *M. paradisiaca* spathe represents a sustainable and underutilized botanical resource with considerable potential for the development of nutraceutical and phytopharmaceutical interventions for iron deficiency-associated anaemia. Further studies are warranted to

identify the active constituents, elucidate the underlying molecular mechanisms, and validate its clinical efficacy.

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7. Author Contributions
Rajeswar Das: Conceptualization, methodology, investigation, formal analysis, data curation, visualization, writing original draft preparation. Durgaprasad Kemisetti: Conceptualization, supervision, methodology, validation, project administration, resources, writing review and editing, corresponding author. Bolay Bhattacharya: Supervision, methodology, validation, resources, writing review and editing. All authors have read and approved the final manuscript and accept responsibility for the integrity and accuracy of the work.

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