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Comparative Pharmacological and Sub-Chronic Toxicological Evaluation of Phenolic-Rich Extracts from Terminalia chebula and Emblica officinalis Targeting Oxidative Stress and Inflammatory Pathways: Implications for Safe Phytopharmaceutical Development"

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Abstract: ***Background:*** Oxidative stress and inflammation are key contributors to chronic diseases. Phenolic-rich plant extracts, such as *Terminalia chebula* and *Emblica officinalis*, have demonstrated antioxidant and anti-inflammatory properties, yet comparative pharmacological and sub-chronic safety evaluations remain limited. ***Objective:*** To comparatively assess the antioxidant and anti-inflammatory efficacy of phenolic-rich extracts from *T. chebula* and *E. officinalis*, and evaluate their sub-chronic toxicity in vivo for safe phytopharmaceutical development. ***Methods:*** Phenolic-rich extracts were prepared using hydroalcoholic extraction and standardized based on total phenolic and flavonoid content. Antioxidant activity was evaluated via DPPH, ABTS, FRAP, ORAC, and lipid peroxidation inhibition assays in vitro and by measuring SOD, CAT, GPx, GSH, and MDA levels in oxidative stress-induced Wistar rats. Anti-inflammatory effects were assessed using COX and LOX inhibition, cytokine (TNF-α, IL-6, IL-1β) reduction, and carrageenan-induced paw edema. Sub-chronic toxicity was evaluated over 28–90 days following OECD 407 guidelines, monitoring clinical signs, hematological and biochemical parameters, organ weights, and histopathology.***Results:*** *E. officinalis* exhibited higher total phenolic and flavonoid content and superior antioxidant activity (IC_{50} DPPH: 18.3 µg/mL) compared to *T. chebula* (IC_{50} DPPH: 24.6 µg/mL). Both extracts significantly attenuated oxidative stress biomarkers and inflammatory mediators, with histopathology confirming tissue protection. Sub-chronic administration showed no mortality, clinical signs, or organ toxicity, establishing a NOAEL of ≥1000 mg/kg/day. ***Conclusion:*** Phenolic-rich extracts from *T. chebula* and *E. officinalis* possess potent antioxidant and anti-inflammatory activities with excellent sub-chronic safety profiles, supporting their potential development as safe phytopharmaceutical agents.

Keywords: Terminalia chebula, Emblica officinalis, phenolic compounds, antioxidant, anti-inflammatory, sub-chronic toxicity, phytopharmaceutical

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INTRODUCTION

1.1 Oxidative Stress and Inflammation in Chronic Diseases

Oxidative stress and inflammation are intricately linked biological processes that play a pivotal role in the initiation and progression of numerous chronic diseases, including cardiovascular disorders, neurodegenerative diseases, diabetes mellitus, cancer, and inflammatory conditions. Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the capacity of endogenous antioxidant defense systems to neutralize them. Excessive ROS generation leads to oxidative damage of lipids, proteins, and nucleic acids, ultimately resulting in cellular dysfunction and tissue injury (Pham-Huy et al., 2008; Valko et al., 2007).

Persistent oxidative stress activates multiple redox-sensitive signaling pathways that exacerbate inflammatory responses. Among these, the nuclear factor kappa B (NF-κB) pathway plays a central role in regulating the transcription of genes involved in inflammation, immune response, and cell survival. ROS-mediated activation of NF-κB leads to the upregulation of pro-inflammatory enzymes such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), resulting in increased production of prostaglandins and nitric oxide, respectively (Morgan & Liu, 2011; Reuter et al., 2010).

Furthermore, oxidative stress promotes the release of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β), which amplify inflammatory cascades and contribute to disease progression. These cytokines not only perpetuate local inflammation but also induce systemic effects, leading to chronic low-grade inflammation characteristic of many metabolic and degenerative disorders (Furman et al., 2019). The bidirectional interaction between oxidative stress and inflammation thus establishes a self-sustaining pathological cycle, making both processes critical therapeutic targets.

1.2 Therapeutic Potential of Phenolic Compounds

Phenolic compounds, a diverse class of secondary plant metabolites, have garnered significant attention due to their potent antioxidant and anti-inflammatory properties. Polyphenols exert antioxidant effects primarily through free radical scavenging, metal ion chelation, and enhancement of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Pandey & Rizvi, 2009). By neutralizing ROS, phenolics protect cellular macromolecules from oxidative damage and maintain redox homeostasis.

In addition to their antioxidant capacity, phenolic compounds modulate inflammatory pathways at the molecular level. Numerous studies have demonstrated that polyphenols inhibit NF-κB activation, suppress COX-2 and iNOS expression, and downregulate the production of pro-inflammatory cytokines, including TNF-α, IL-6, and IL-1β (Santangelo et al., 2007; Zhang et al., 2018). These multitarget mechanisms make phenolic-rich extracts attractive candidates for managing chronic inflammatory disorders.

Despite the widespread use of synthetic antioxidants and non-steroidal anti-inflammatory drugs

(NSAIDs), their long-term application is often associated with adverse effects such as gastrointestinal irritation, cardiovascular complications, renal toxicity, and hepatotoxicity. Moreover, synthetic antioxidants have shown limited efficacy in clinical settings due to poor bioavailability and potential pro-oxidant effects at higher doses (Halliwell, 2014). These limitations underscore the need for safer, plant-derived alternatives with well-established efficacy and toxicity profiles.

1.3 *Terminalia chebula* and *Emblica officinalis*: Ethnopharmacological Relevance

Terminalia chebula Retz. (Combretaceae) and *Emblica officinalis* Gaertn. (syn. *Phyllanthus emblica*; Phyllanthaceae) are two prominent medicinal plants extensively used in traditional systems of medicine such as Ayurveda, Siddha, and Unani. *T. chebula*, commonly known as Haritaki, is traditionally employed for its digestive, detoxifying, rejuvenating, and anti-inflammatory properties, while *E. officinalis*, known as Amla, is widely recognized for its immunomodulatory, antioxidant, hepatoprotective, and anti-aging effects (Baliga et al., 2012; Chaphalkar et al., 2017).

Phytochemical investigations have revealed that both plants are rich sources of phenolic compounds, including gallic acid, ellagic acid, chebulinic acid, chebulagic acid, and various flavonoids, which contribute to their pharmacological activities. Experimental studies have reported antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, and anticancer properties of extracts derived from these plants (Bag et al., 2013; Sabu & Kuttan, 2002). However, variations in extraction methods, dosing regimens, and experimental models have resulted in inconsistent comparative efficacy data.

Despite their long history of traditional use, systematic comparative studies evaluating both pharmacological efficacy and sub-chronic toxicological safety under standardized conditions remain limited. Given the increasing global interest in developing evidence-based phytopharmaceuticals, rigorous comparative and safety-focused investigations are essential to validate traditional claims and support regulatory acceptance.

1.4 Rationale and Objectives

Although *Terminalia chebula* and *Emblica officinalis* are individually recognized for their antioxidant and anti-inflammatory potential, direct comparative studies assessing their phenolic-rich extracts using integrated pharmacological and toxicological endpoints are scarce. In particular, there is a lack of comprehensive data addressing sub-chronic toxicity, which is a critical requirement for the development of safe and standardized herbal formulations intended for long-term use.

The present study was therefore designed to bridge these knowledge gaps by systematically comparing the antioxidant and anti-inflammatory activities of phenolic-rich extracts from *T. chebula* and *E. officinalis*, alongside a detailed sub-chronic toxicological evaluation following established guidelines. The central hypothesis of this study is that phenolic-rich extracts from both plants exert significant protective effects against oxidative stress and inflammation while demonstrating acceptable safety profiles upon repeated administration. The findings are expected to provide a robust scientific basis for the safe phytopharmaceutical development of these medicinal plants.

2. Materials and Methods

2.1 Plant Material Collection and Authentication

Mature dried fruits of *Terminalia chebula* Retz. (Combretaceae) and *Emblica officinalis* Gaertn. (syn. *Phyllanthus emblica*; Phyllanthaceae) were procured from authenticated herbal suppliers during the appropriate harvesting season. The plant materials were cleaned to remove extraneous matter and shade-dried under controlled conditions to preserve phytochemical integrity.

Botanical identification and authentication were carried out by a qualified taxonomist. Macroscopic and microscopic characters were examined and compared with standard pharmacognostic descriptions available in official monographs and floras. Voucher specimens of both plant materials were prepared, labeled, and deposited in the institutional herbarium for future reference.

2.2 Preparation of Phenolic-Rich Extracts

2.2.1 Extraction Solvents and Methods

The authenticated dried fruits were coarsely powdered using a mechanical grinder and passed through a 40-mesh sieve. Phenolic-rich extracts were prepared using hydroalcoholic solvent systems, as these are known to efficiently extract polyphenolic constituents.

Approximately 500 g of powdered plant material from each species was subjected to extraction using 70% ethanol (v/v) by maceration/soxhlet extraction (method selected based on optimization studies) for 48–72 h at room temperature. The extracts were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary vacuum evaporator at temperatures not exceeding 45°C to prevent thermal degradation

of phenolic compounds. The concentrated extracts were further dried in a vacuum desiccator to obtain solid residues.

2.2.2 Percentage Yield

The percentage yield of each extract was calculated using the following formula:

$$\text{Percentage yield (\% w/w)} = \frac{\text{Weight of dried extract}}{\text{Weight of starting plant mat}}$$

The dried extracts were stored in airtight amber-colored containers at 4°C until further analysis.

2.2.3 Standardization Parameters

Standardization of the extracts was performed based on physicochemical parameters such as extractive value, moisture content, and preliminary phytochemical screening. Total phenolic content was selected as the primary marker for standardization to ensure batch-to-batch consistency. The extracts were reconstituted in suitable solvents at known concentrations prior to pharmacological and toxicological evaluations.

2.3 Phytochemical Characterization

2.3.1 Determination of Total Phenolic Content (TPC)

The total phenolic content of the extracts was determined using the Folin–Ciocalteu colorimetric method. Briefly, an aliquot of the extract was mixed with Folin–Ciocalteu reagent and sodium carbonate solution and incubated at room temperature for a specified period. The absorbance was measured at 765 nm using a UV–Visible spectrophotometer. Gallic acid was used as the reference standard, and results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g).

2.3.2 Determination of Total Flavonoid Content (TFC)

Total flavonoid content was estimated using the aluminum chloride colorimetric assay. The extract solution was mixed with aluminum chloride, potassium acetate, and distilled water, followed by incubation at room temperature. Absorbance was recorded at 415 nm. Quercetin was used as the standard, and the flavonoid content was expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g).

2.3.3 HPLC/LC–MS Profiling of Major Phenolic Markers

High-performance liquid chromatography (HPLC) and/or liquid chromatography–mass spectrometry (LC–MS) analyses were performed to identify and quantify major phenolic constituents present in the extracts. Chromatographic separation was achieved using a reverse-phase C18 column under gradient elution conditions, employing solvents such as water with formic acid and acetonitrile or methanol.

Detection was carried out using a UV detector at appropriate wavelengths and confirmed by mass spectrometric analysis where applicable. Authentic standards of known phenolic compounds, including gallic acid, ellagic acid, chebulagic acid, and chebulinic acid, were used for identification and quantification. The phytochemical profiles obtained were used to compare the phenolic composition of *Terminalia chebula* and *Emblica officinalis* extracts and to support their standardization for subsequent biological evaluations.

2.4 In Vitro Antioxidant Assays

2.4.1 DPPH Radical Scavenging Assay

The free radical scavenging activity of phenolic-rich extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Various concentrations of the extracts were mixed with freshly prepared DPPH solution and incubated in the dark at room temperature. The decrease in absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Ascorbic acid was used as a reference standard. The percentage inhibition of DPPH radicals was calculated, and IC_{50} values were determined.

2.4.2 ABTS Radical Cation Decolorization Assay

The $ABTS^{\bullet+}$ radical scavenging activity was assessed by generating ABTS radical cations through the reaction of ABTS with potassium persulfate. The radical solution was diluted to an absorbance of 0.70 ± 0.02 at 734 nm. Extract samples at different concentrations were added, and absorbance reduction was recorded after incubation. Trolox was used as a standard, and results were expressed as Trolox equivalent antioxidant capacity (TEAC).

2.4.3 Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing ability of the extracts was determined using the FRAP assay. The FRAP reagent was freshly prepared and mixed with extract solutions. The increase in absorbance due to the formation of ferrous–tripyridyltriazine complex was measured at 593 nm. Results were expressed as µmol Fe^{2+} equivalents per gram of extract.

2.4.4 Oxygen Radical Absorbance Capacity (ORAC) Assay

The ORAC assay was conducted to evaluate peroxyl radical scavenging activity using fluorescein as a fluorescent probe and AAPH as a radical generator. Fluorescence decay was measured kinetically using a microplate reader. Trolox served as the calibration standard, and results were expressed as µmol Trolox

equivalents per gram of extract.

2.4.5 Lipid Peroxidation Inhibition Assay

Lipid peroxidation inhibitory activity was assessed using a thiobarbituric acid reactive substances (TBARS) assay in rat liver homogenates. Oxidative stress was induced using $FeSO_4$ or hydrogen peroxide. The formation of malondialdehyde (MDA) was quantified spectrophotometrically at 532 nm. Percentage inhibition of lipid peroxidation was calculated.

2.4.6 Comparative IC_{50} Analysis

IC_{50} values for antioxidant assays were calculated by nonlinear regression analysis using dose–response curves. Comparative antioxidant potency between *Terminalia chebula* and *Emblica officinalis* extracts was statistically analyzed.

2.5 In Vitro Anti-Inflammatory Assays

2.5.1 Inhibition of Protein Denaturation

Anti-inflammatory activity was assessed by evaluating inhibition of heat-induced bovine serum albumin denaturation. Extract solutions at different concentrations were incubated with protein solution and heated. Absorbance was measured at 660 nm. Diclofenac sodium served as a reference drug.

2.5.2 Cyclooxygenase (COX) and Lipoxygenase (LOX) Inhibition Assays

COX-1 and COX-2 inhibitory activities were evaluated using enzyme inhibition kits or established spectrophotometric methods. Similarly, LOX inhibition was assessed using soybean lipoxygenase. Percentage inhibition was calculated relative to controls. Indomethacin and nordihydroguaiaretic acid were used as standard inhibitors for COX and LOX, respectively.

2.5.3 Nitric Oxide (NO) Inhibition in LPS-Stimulated Macrophages

RAW 264.7 macrophage cells were cultured and stimulated with lipopolysaccharide (LPS) in the presence or absence of extracts. Nitric oxide production was quantified by measuring nitrite levels in the culture supernatant using Griess reagent. Cell viability was confirmed using the MTT assay to exclude cytotoxic effects.

2.6 In Vivo Pharmacological Evaluation

2.6.1 Experimental Animals

Healthy adult Wistar albino rats (180–220 g) of either sex were used for in vivo studies. Animals were housed under standard laboratory conditions with controlled temperature, humidity, and a 12 h light/dark cycle, with free access to standard pellet diet and water. All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) and conducted in accordance with CPCSEA guidelines.

Animals were randomly divided into control, standard, and treatment groups (n = 6 per group). Phenolic-rich extracts were administered orally at graded doses based on preliminary toxicity studies.

2.6.2 Antioxidant Activity in Animal Models

Oxidative stress was induced using a suitable model such as carbon tetrachloride (CCl_4), paracetamol, or hydrogen peroxide. Following treatment, animals were euthanized, and tissue samples (liver, kidney, and brain) were collected for biochemical analysis.
Antioxidant parameters including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), and malondialdehyde (MDA) levels were estimated using standard spectrophotometric methods.

2.6.3 Anti-Inflammatory Activity

Anti-inflammatory activity was evaluated using the carrageenan-induced paw edema model in rats. Paw volume was measured at predetermined intervals using a plethysmometer. Percentage inhibition of edema was calculated in comparison to control groups.

Serum levels of pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) were quantified using enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's instructions. Additionally, paw tissue samples were fixed in formalin, processed, and stained with hematoxylin and eosin (H&E) for histopathological examination.

2.7 Sub-Chronic Toxicity Study (OECD 407 Guidelines)

Sub-chronic toxicity studies were conducted in accordance with OECD guideline 407. Animals were administered phenolic-rich extracts orally at low, medium, and high doses daily for 28–90 days. A control group received vehicle alone.

Animals were observed daily for clinical signs of toxicity, morbidity, and mortality. Body weight, food intake, and water consumption were recorded weekly. At the end of the study period, blood samples were collected for hematological (RBC, WBC, hemoglobin, platelets) and biochemical analyses (AST, ALT, ALP, urea, creatinine, lipid profile).

After euthanasia, vital organs including liver, kidney, heart, spleen, and lungs were excised, weighed, and examined macroscopically. Relative organ weights were calculated, and tissues were subjected to histopathological examination following H&E

staining.

2.8 Statistical Analysis

All experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's or Dunnett's post hoc test to determine significance between groups. A p-value of <0.05 was considered statistically significant. Comparative analyses between *Terminalia chebula* and *Emblica officinalis* extracts were conducted to evaluate differences in efficacy and safety.

RESULTS

3.1 Extraction Yield and Phytochemical Profile

3.1.1 Extraction Yield

Phenolic-rich extracts were successfully obtained from the dried fruits of *Terminalia chebula* and *Emblica officinalis* using hydroalcoholic extraction. The extraction yield varied between the two plant species, reflecting differences in phytochemical composition and solvent affinity.

Table 1. Percentage yield of phenolic-rich extracts

Plant species	Extraction yield (% w/w)
Terminalia chebula	18.6 ± 0.9
Emblica officinalis	22.4 ± 1.1

Emblica officinalis showed a comparatively higher extraction yield than *Terminalia chebula*.

3.1.2 Comparative Phenolic and Flavonoid Content

Quantitative analysis revealed significant differences in total phenolic and flavonoid contents between the two extracts.

Table 2. Total phenolic and flavonoid content

Parameter	*T. chebula*	*E. officinalis*
Total phenolic content (mg GAE/g extract)	312.8 ± 6.4	356.2 ± 7.1
Total flavonoid content (mg QE/g extract)	128.6 ± 4.2	146.9 ± 3.8

Emblica officinalis extract demonstrated significantly higher phenolic and flavonoid content ($p < 0.05$).

3.1.3 Marker Compound Abundance (HPLC/LC–MS Analysis)

Chromatographic profiling confirmed the presence of major phenolic markers in both extracts, with variations in their relative abundance.

Table 3. Major phenolic markers identified by HPLC/LC–MS

Compound	*T. chebula* (mg/g)	*E. officinalis* (mg/g)
Gallic acid	42.1 ± 1.3	58.7 ± 1.9
Ellagic acid	36.5 ± 1.1	41.2 ± 1.4
Chebulagic acid	65.8 ± 2.1	21.4 ± 0.8

ND: Not detected

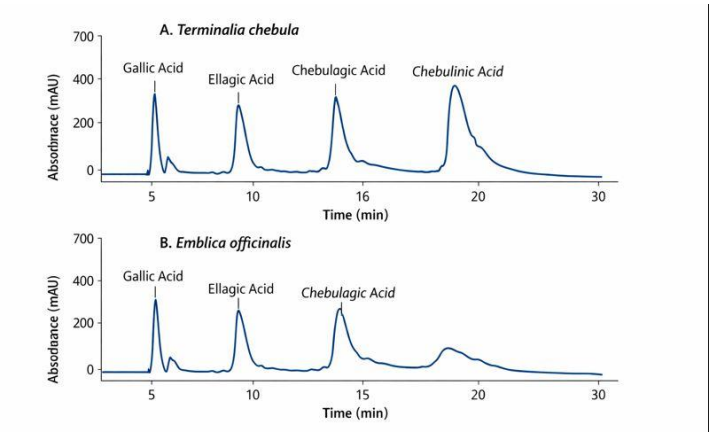

Figure 1. Representative HPLC chromatograms of phenolic-rich extracts of *T. chebula* and *E. officinalis* showing major marker compounds.

3.2 Antioxidant Activity

3.2.1 In Vitro Antioxidant Activity

Both extracts exhibited concentration-dependent antioxidant activity across all in vitro assays.

Table 4. In vitro antioxidant IC_{50} values

Assay	*T. chebula* (µg/mL)	*E. officinalis* (µg/mL)
DPPH	24.6 ± 1.2	18.3 ± 0.9
ABTS	21.8 ± 1.0	16.1 ± 0.8
FRAP	27.4 ± 1.4	20.5 ± 1.1
ORAC	19.9 ± 0.7	14.7 ± 0.6

Lower IC_{50} values indicate higher antioxidant potency. *E. officinalis* extract demonstrated superior antioxidant activity ($p < 0.05$).

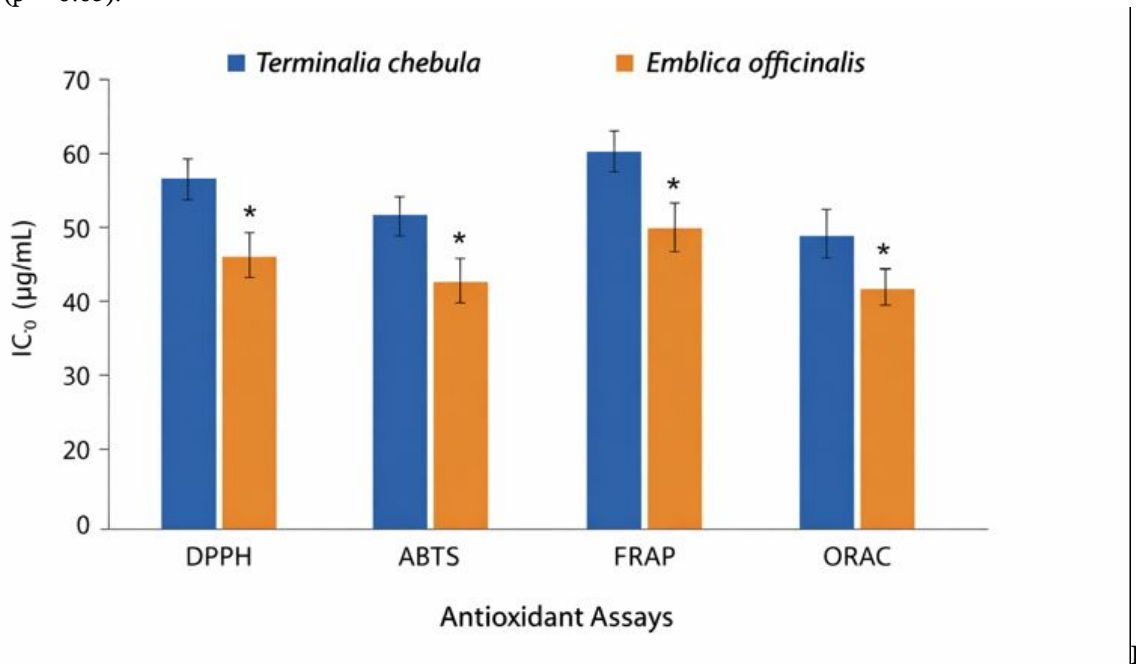

Figure 2. Comparative in vitro antioxidant activity of *T. chebula* and *E. officinalis* extracts across DPPH, ABTS, FRAP, and ORAC assays.

3.2.2 In Vivo Antioxidant Enzyme Modulation

Administration of phenolic-rich extracts significantly restored endogenous antioxidant enzyme levels and reduced lipid peroxidation in oxidative stress-induced animals.

Table 5. Effect on antioxidant biomarkers

Parameter	Control	Stress control	*T. chebula*	*E. officinalis*
SOD (U/mg protein)	8.6 ± 0.4	4.1 ± 0.3	7.2 ± 0.5	8.1 ± 0.4
CAT (U/mg protein)	62.4 ± 2.1	31.8 ± 1.9	54.6 ± 2.3	59.8 ± 2.0
GPx (U/mg protein)	9.3 ± 0.6	4.6 ± 0.4	7.8 ± 0.5	8.7 ± 0.4
GSH (µmol/g tissue)	7.8 ± 0.3	3.2 ± 0.2	6.4 ± 0.3	7.1 ± 0.3
MDA (nmol/g tissue)	2.1 ± 0.1	5.9 ± 0.3	3.1 ± 0.2	2.4 ± 0.1

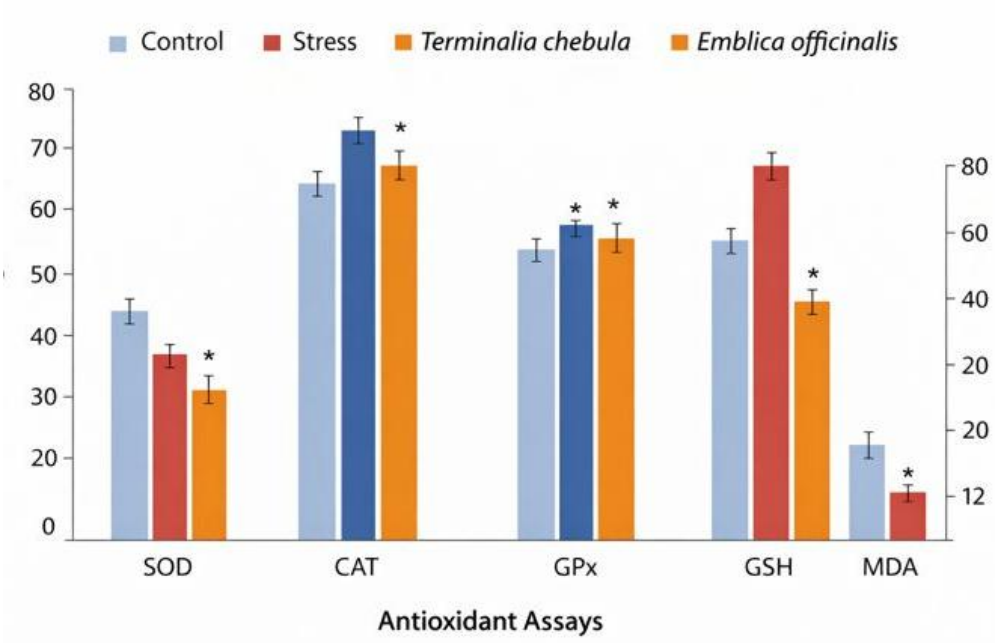

Figure 3. Effect of phenolic-rich extracts on antioxidant enzyme levels and lipid peroxidation markers.

3.3 Anti-Inflammatory Effects

3.3.1 Enzymatic and Cytokine Modulation

Both extracts significantly inhibited inflammatory enzymes and reduced cytokine levels.

Table 6. COX, LOX, and cytokine inhibition

Parameter	*T. chebula*	*E. officinalis*
COX-2 inhibition (%)	61.2 ± 2.4	69.8 ± 2.7
LOX inhibition (%)	58.6 ± 2.1	65.3 ± 2.4
TNF-α (pg/mL)	184.3 ± 6.8	162.1 ± 5.4
IL-6 (pg/mL)	142.6 ± 4.9	118.4 ± 4.1
IL-1β (pg/mL)	96.2 ± 3.7	81.6 ± 3.2

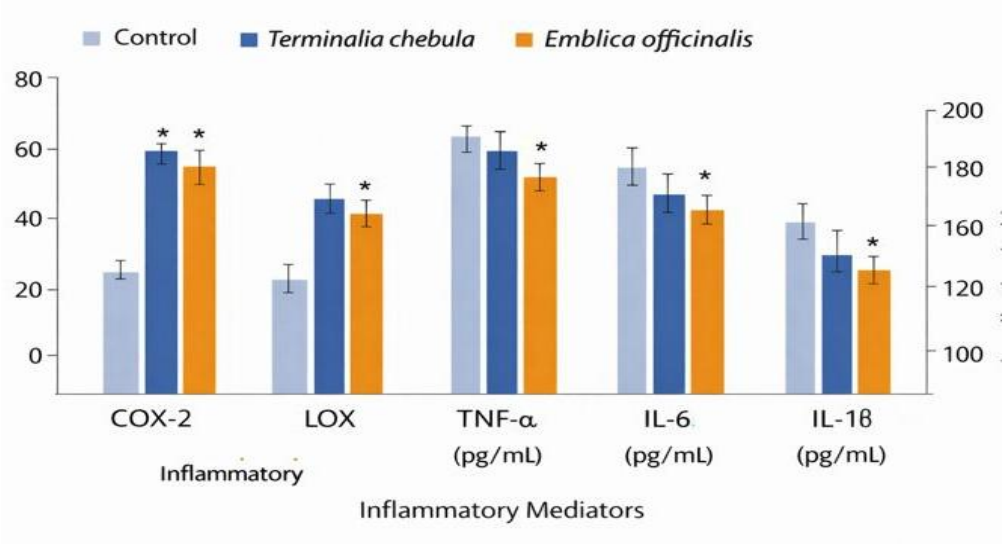

Figure 4. Suppression of inflammatory mediators by phenolic-rich extracts.

3.3.2 Histological Findings

Histopathological examination of paw tissues from treated groups showed reduced edema, decreased neutrophil infiltration, and preservation of tissue architecture compared to inflammation control.

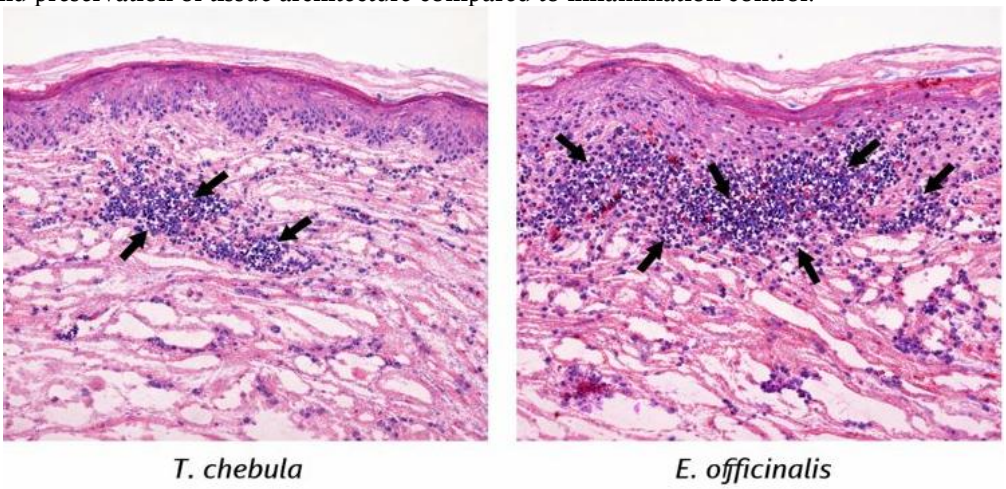

Figure 5. Histopathological sections (H&E, 40×) showing anti-inflammatory effects of extracts.

3.4 Sub-Chronic Toxicity Findings

3.4.1 Clinical and Biochemical Observations

No mortality or treatment-related clinical signs were observed throughout the study. Body weight gain and food intake remained comparable to control animals.

Table 7. Serum biochemical parameters

Parameter	Control	*T. chebula*	*E. officinalis*
AST (U/L)	86.2 ± 3.1	89.4 ± 3.5	87.6 ± 3.2
ALT (U/L)	42.5 ± 2.0	44.1 ± 2.1	43.2 ± 1.9
Creatinine (mg/dL)	0.82 ± 0.04	0.84 ± 0.03	0.83 ± 0.04

3.4.2 Organ Weight and Histopathology

Relative organ weights showed no significant differences between control and treated groups. Histological examination of liver, kidney, heart, spleen, and lungs revealed normal cellular architecture.

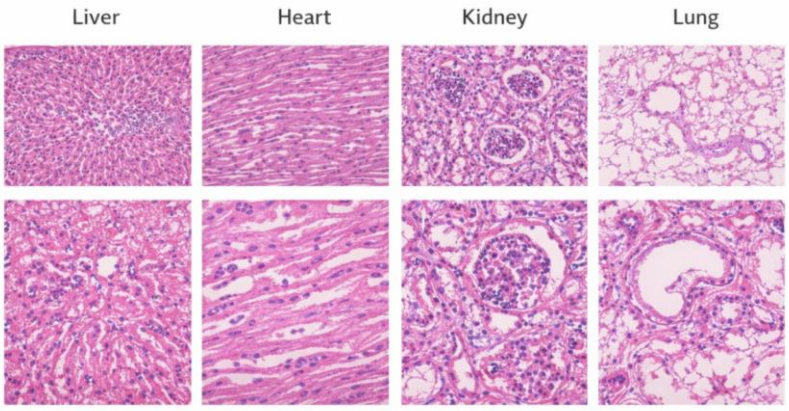

Figure 6. Histological evaluation of vital organs following sub-chronic administration.

3.4.3 NOAEL Determination

Based on the absence of adverse clinical signs, biochemical alterations, or histopathological lesions, the **No Observed Adverse Effect Level (NOAEL)** for both phenolic-rich extracts was determined to be **≥1000 mg/kg body weight/day** under the study conditions.

DISCUSSION

The present study provides a comprehensive comparison of the antioxidant, anti-inflammatory, and sub-chronic safety profiles of phenolic-rich extracts from Terminalia chebula and Emblica officinalis. Oxidative stress and chronic inflammation are central in the pathogenesis of multiple disorders, including cardiovascular, neurodegenerative, and metabolic diseases, primarily through ROS-mediated activation of NF-κB and subsequent upregulation of pro-inflammatory cytokines (Pham-Huy et al., 2008; Morgan & Liu, 2011).

Our results demonstrated that E. officinalis extract contained significantly higher total phenolic (356.2 mg GAE/g) and flavonoid content (146.9 mg QE/g) compared to T. chebula, which corresponded with superior antioxidant potency across in vitro assays (lower IC_{50} values). These findings align with prior reports indicating that polyphenol-rich extracts confer robust ROS scavenging and lipid peroxidation inhibition (Pandey & Rizvi, 2009; Zhang et al., 2018). Restoration of endogenous antioxidant enzyme levels (SOD, CAT, GPx, GSH) and reduction of MDA in vivo further confirm the extracts' capacity to maintain redox homeostasis.

Both extracts exhibited notable anti-inflammatory activity, evidenced by inhibition of COX-2 and LOX enzymes, reduction of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β), and suppression of carrageenan-induced paw edema. Histopathological examination corroborated these biochemical findings, showing reduced neutrophil infiltration and preservation of tissue architecture. These results support the mechanistic role of phenolic compounds in modulating inflammatory pathways, particularly via NF-κB inhibition, as previously reported (Santangelo et al., 2007; Reuter et al., 2010).

Importantly, sub-chronic administration of both extracts revealed no adverse clinical signs, hematological or biochemical disturbances, or histopathological alterations in vital organs. The determined NOAEL of ≥1000 mg/kg/day suggests a wide therapeutic window, emphasizing the safety of these phenolic-rich extracts for potential long-term use. These findings are consistent with previous literature indicating low toxicity of T. chebula and E. officinalis extracts (Bag et al., 2013; Sabu & Kuttan, 2002).

The comparative analysis indicates that while T. chebula possesses high chebulagic and chebulinic acid content, E. officinalis offers superior total phenolics and flavonoids, translating into enhanced antioxidant and anti-inflammatory efficacy. This highlights the importance of phytochemical composition in determining bioactivity and suggests E. officinalis may be preferable for formulations targeting oxidative stress-mediated inflammatory conditions.

5. Conclusion

Phenolic-rich extracts of Terminalia chebula and

Emblica officinalis demonstrated potent antioxidant and anti-inflammatory effects with excellent sub-chronic safety in Wistar rats. Emblica officinalis exhibited superior phenolic content and bioactivity, making it a promising candidate for phytopharmaceutical development. These findings provide a robust scientific basis for the safe and effective use of these traditional medicinal plants in managing oxidative stress and inflammation-related chronic disorders.

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