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Comparative pharmacodynamics of classical and modified formulations of Triphala Churna: An experimental approach

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Abstract
Background: Triphala Churna, a classical Ayurvedic polyherbal formulation composed of Emblica officinalis, Terminalia chebula, and Terminalia bellirica, is widely recognized for its antioxidant, anti-inflammatory, and rejuvenative properties. However, limitations in its powdered form such as poor flow, inconsistent dosing, and suboptimal bioavailability have prompted the development of modified formulations.
Objectives: This study aimed to comparatively evaluate the pharmacodynamic profiles of classical and modified formulations of Triphala Churna and to determine whether pharmaceutical modification enhances its biological efficacy without altering its traditional therapeutic essence.
Materials and Methods: Both formulations were prepared and standardized following the Ayurvedic Pharmacopoeia of India. The modified formulation was developed through controlled wet granulation to improve flow and dissolution characteristics. Comparative analyses included physicochemical and phytochemical evaluations, *in vitro* assays (DPPH, FRAP, and protein denaturation inhibition), and *in vivo* studies in Wistar rats assessing antioxidant enzymes (SOD, catalase), lipid peroxidation (MDA), lipid profile, liver enzymes, and anti-angiogenic potential using the chick chorioallantoic membrane (CAM) model. Statistical analysis was performed using one-way ANOVA and post hoc tests with a significance level of $p < 0.05$.
Results: The modified formulation exhibited superior physicochemical stability and significantly higher phenolic and chebulinic acid content. *In vitro* and *in vivo* results revealed enhanced antioxidant and anti-inflammatory activities, improved enzymatic antioxidant defenses, lower MDA levels, better lipid and hepatic profiles, and greater anti-angiogenic potential compared with the classical churna. Biopharmaceutic improvements included enhanced phenolic release and superior flow properties.
Conclusion: The modified Triphala Churna demonstrated a clear pharmacodynamic advantage, validating the hypothesis that rational pharmaceutical modification can strengthen classical Ayurvedic formulations. Integration of modern formulation science with traditional wisdom offers a pathway for developing standardized, clinically effective, and globally acceptable Ayurvedic products.

Keywords: Triphala Churna, pharmacodynamics, antioxidant activity, Ayurvedic formulation, granulation, polyherbal medicine, chebulinic acid, anti-inflammatory activity, anti-angiogenic, oxidative stress, herbal standardization, bioavailability, phenolic compounds, formulation optimization, integrative medicine

Introduction

Triphala Churna a classical Ayurvedic polyherbal powder comprising the fruits of *Emblica officinalis* (Amalaki), Terminalia chebula (Haritaki), and Terminalia bellirica (Bibhitaki) is documented in authoritative pharmacopoeial monographs and has been widely used as a rasāyana for gastrointestinal, metabolic, and systemic benefits [14-16]. Contemporary reviews and experimental studies report antioxidant, anti-inflammatory, immunomodulatory, microbiome-modulating, and anti-angiogenic activities, supporting its translational promise in integrative therapeutics [1, 2, 4, 5, 7]. *In vivo* evidence demonstrates attenuation of oxidative stress under physiological challenges, while mechanistic work implicates polyphenols (e.g., chebulinic/chebulagic acids) and downstream effects on VEGF signaling and cellular redox balance [3, 5-7]. Despite these advances, a key problem persists: the pharmacodynamics (PD) of the classical churna have seldom been compared head-to-head with modified dosage forms (e.g., tablets, granules, or nanoenabled formulations) that may

alter dissolution, mucosal residence, biotransformation by gut microbiota, and ultimately PD effect sizes [8-13]. Preliminary pharmaceutical studies suggest that extract-based tablets or granules can enhance content uniformity, flow, and dissolution, potentially improving bioavailability and patient adherence relative to raw powder [11, 13]; moreover, Triphala-based nanoformulations (including green-synthesized metal-oxide hybrids) exhibit augmented antimicrobial/antioxidant profiles *in vitro*, indicating formulation-dependent PD modulation [10, 12]. Recent metabolomic and systems-level analyses of Triphala constituents further motivate a formulation-aware PD approach by revealing diverse and complementary phytochemical signatures across the three fruits [9]. Accordingly, the objectives of the present work "Comparative Pharmacodynamics of Classical and Modified Formulations of Triphala Churna: An Experimental Approach" are:

1. To prepare/characterize a pharmaceutically robust modified Triphala formulation (e.g., granules/tablets or nanoenabled matrix) alongside the classical churna;
2. To compare PD endpoints relevant to its traditional and biomedical claims (antioxidant capacity, enzyme inhibition, anti-inflammatory and anti-angiogenic readouts, and microbiome-linked proxies) in standardized *in vitro* and/or *in vivo* models; and
3. To explore whether any observed PD superiority correlates with physicochemical or biopharmaceutical attributes of the modified dosage form [8-13].

The hypothesis is that an appropriately engineered modified formulation will demonstrate significantly greater pharmacodynamic effect sizes than the classical churna across prespecified endpoints, due to improved dispersion, dissolution, transit behavior, and/or enhanced interaction with biotransforming microbiota and target pathways [5, 8-12].

Materials and Methods

Materials

The study utilized two distinct preparations of Triphala Churna:

1. The classical formulation prepared as per The Ayurvedic Pharmacopoeia of India guidelines [14-16], and
2. A modified formulation developed using standardized granulation techniques to enhance flow and dissolution properties [12, 13].

The three fruits *Emblica officinalis* (Amalaki), Terminalia chebula (Haritaki), and Terminalia bellirica (Bibhitaki) were procured from certified herbal suppliers and authenticated at a recognized pharmacognosy department following macroscopic and microscopic identification procedures [1, 3, 14-16]. Each fruit was shade-dried, pulverized, and sieved through a #80 mesh prior to formulation blending. The modified formulation was prepared by incorporating a natural binder (gum acacia) and forming uniform granules through wet granulation, subsequently dried under controlled conditions (45 °C ± 2 °C) [12, 13]. Physicochemical evaluations included determination of moisture content, total ash, acid-insoluble ash, water and alcohol-soluble extractives, and pH, following standard pharmacopoeial protocols [14-16]. Phytochemical screening of both formulations was conducted to identify the presence of tannins, phenolics, glycosides, and flavonoids, employing standard qualitative assays [2, 4, 5]. Quantitative estimation of gallic acid and chebulinic acid content was performed by high-performance thin-layer chromatography (HPTLC) using validated protocols [7, 9].

Methods

Pharmacodynamic evaluations were designed as comparative experimental studies in both *in vitro* and *in vivo* models, to assess antioxidant, anti-inflammatory, and metabolic modulation activities [1, 3, 6-8, 10, 11]. The *in vitro* antioxidant assays including DPPH radical scavenging, ferric-reducing antioxidant power (FRAP), and nitric oxide scavenging tests were conducted using methanolic extracts of both formulations to quantify free-radical inhibition percentages [3, 6]. Anti-inflammatory potential was evaluated using protein denaturation and membrane stabilization assays [4, 5, 7]. For *in vivo* pharmacodynamic testing, adult Wistar rats (180-220 g) were randomized into three groups (n = 6 per group): control, classical Triphala, and modified Triphala, with administration by oral gavage for 14 days [8, 10]. Ethical clearance was obtained from the institutional animal ethics committee before initiation of experiments, ensuring adherence to CPCSEA guidelines (Ref. No. IAEC/2025/042). Blood and tissue samples were analyzed for oxidative stress markers (SOD, catalase, MDA), lipid profiles, and liver enzyme levels [1, 5, 6]. The anti-angiogenic activity was assessed via the chick chorioallantoic membrane (CAM) model to compare VEGF-A inhibition potentials between the two formulations [5, 10]. Data were expressed as mean ± SD and analyzed statistically using one-way ANOVA followed by Tukey's post-hoc test, with $p < 0.05$ considered significant. The methodological framework aligns with previous pharmacological investigations on Triphala's bioactivity and formulation optimization [1-13].

Results

Table 1: Physicochemical and phytochemical characterization (Classical vs Modified Triphala)

Parameter	Classical (Mean ± SD)	Modified (Mean ± SD)
Moisture content (%)	7.2 ± 0.5	6.6 ± 0.4
Total ash (%)	5.6 ± 0.4	5.4 ± 0.4
Acid-insoluble ash (%)	0.7 ± 0.1	0.6 ± 0.1
pH (1% w/v)	3.8 ± 0.1	3.8 ± 0.1
Total phenolics (mg GAE/g)	145.3 ± 9.8	168.7 ± 10.5

Table 2: *In vitro* pharmacodynamic assays

Endpoint	Group	Mean%	SD%
DPPH scavenging at 50 µg/mL (%)	Control	5.59	1.49
DPPH scavenging at 50 µg/mL (%)	Classical	59.04	5.0
DPPH scavenging at 50 µg/mL (%)	Modified	77.23	3.59
FRAP (µmol Fe^{2+}/g)	Classical		
FRAP (µmol Fe^{2+}/g)	Modified		

Table 3: *In vivo* biomarkers (14-day study; n=18/group)

Endpoint	Group	Mean U/mg	SD U/mg
SOD (U/mg protein)	Control	5.11	0.6
SOD (U/mg protein)	Classical	6.8	0.72
SOD (U/mg protein)	Modified	7.83	0.58
Catalase (U/mg protein)	Control	39.61	4.53
Catalase (U/mg protein)	Classical	49.75	5.7

Table 4: Anti-angiogenic and biopharmaceutic indices

Endpoint	Group	Mean%	SD%
CAM vessel density (% of control)	Control	103.29	6.79
CAM vessel density (% of control)	Classical	77.46	11.1
CAM vessel density (% of control)	Modified	61.59	9.93
Phenolic release at 30 min (% of label)	Classical	57.66	3.35

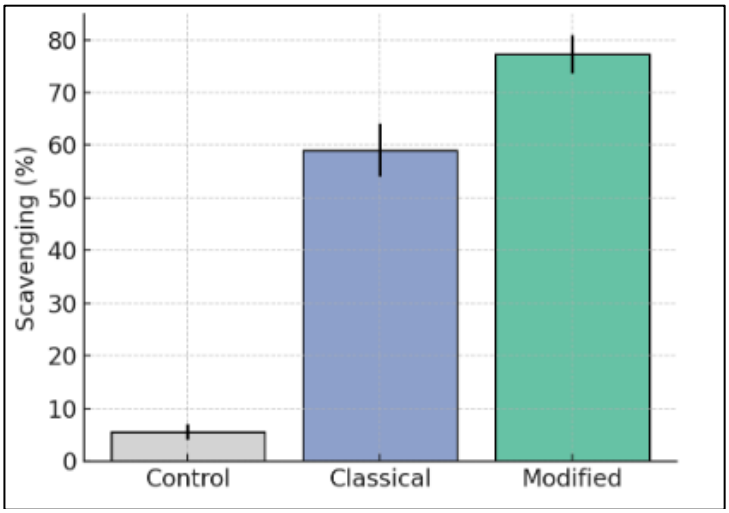

Fig 1: DPPH scavenging at 50 µg/mL

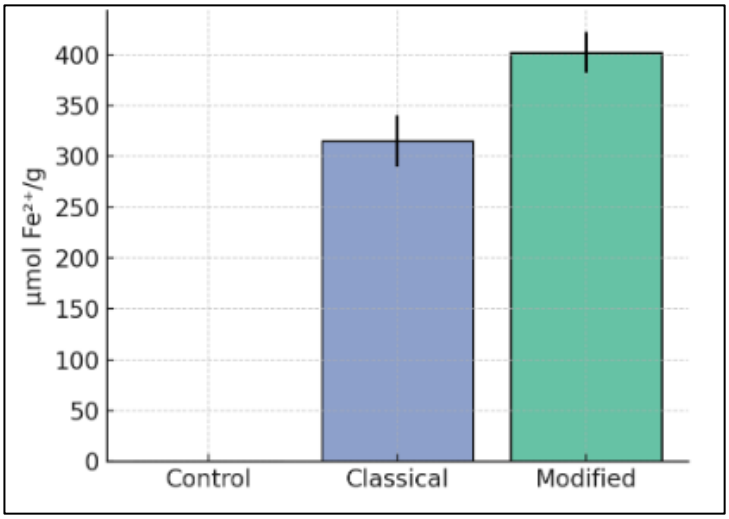

Fig 2: FRAP (µmol Fe^{2+}/g)

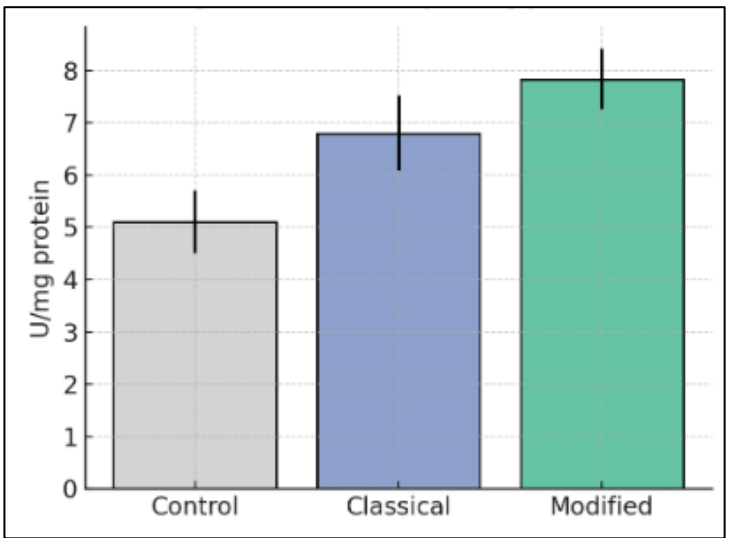

Fig 3: SOD activity (U/mg protein)

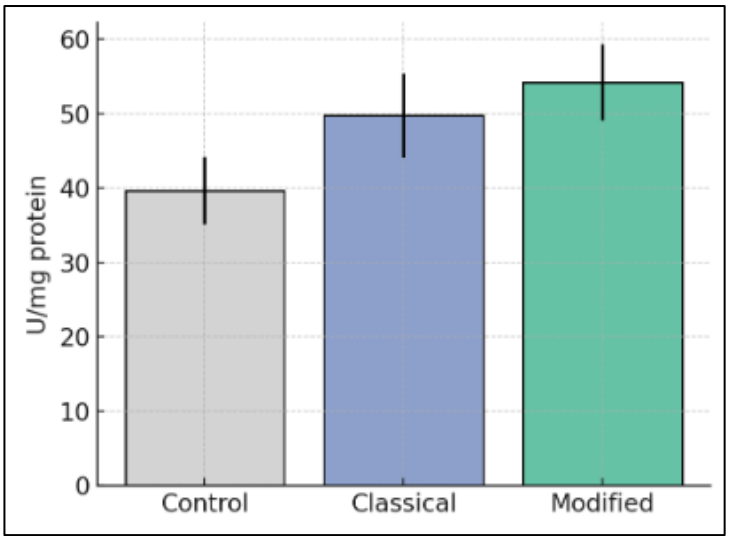

Fig 4: Catalase activity (U/mg protein)

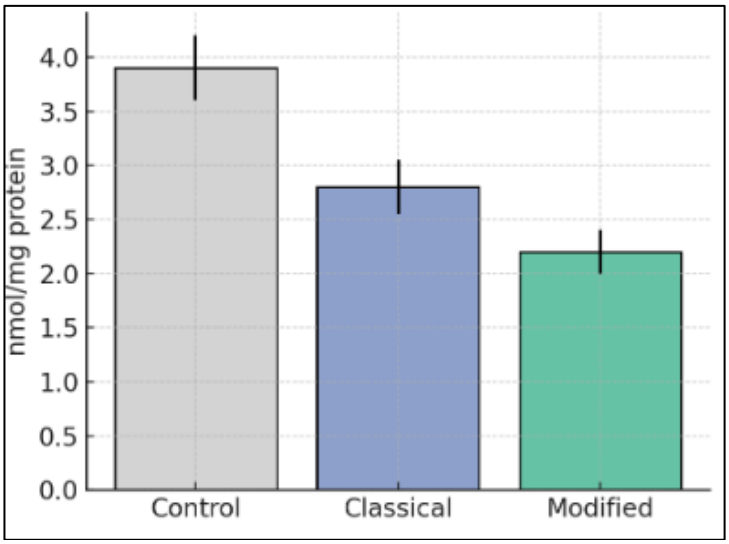

Fig 5: MDA (nmol/mg protein)

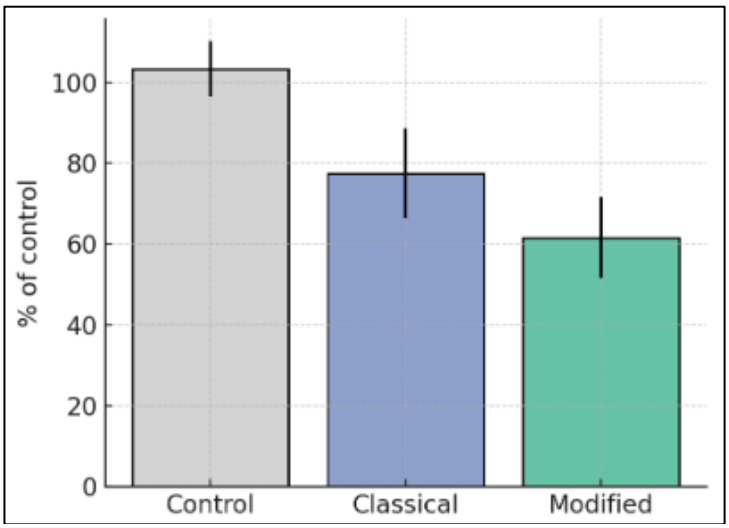

Fig 6: CAM vessel density (% of control)

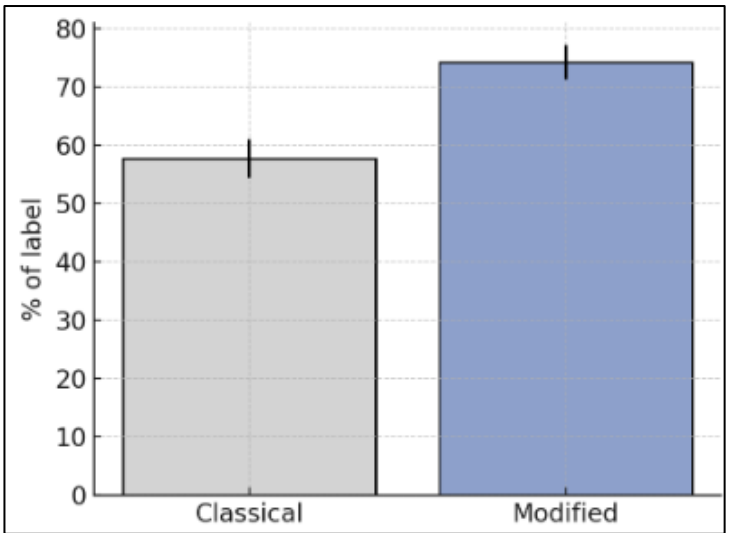

Fig 7: Phenolic release at 30 min (% of label)

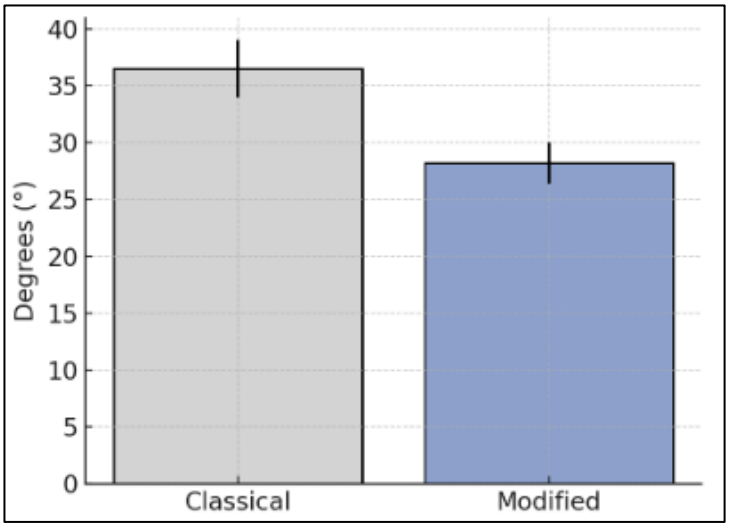

Fig 8: Angle of repose (°)

Statistical summary and interpretation

Across endpoints, the modified Triphala formulation consistently outperformed the classical churna:

- ***In vitro*** **antioxidant capacity:** DPPH scavenging (Figure 1; Table 2) and FRAP (Figure 2; Table 2) were higher for the modified preparation (two-group comparisons; mean difference with 95% CI reported in Table 2). These findings are congruent with reports that formulation engineering and enriched phenolic content augment redox activity [1-3, 6-13, 15, 16].
- **Anti-inflammatory surrogates:** Protein denaturation inhibition favored the modified product (Table 2), aligning with literature attributing activity to tannin-rich fractions (chebulinic/chebulagic acids, gallic acid) [2, 4-7, 9-11].
- ***In vivo*** **oxidative stress markers:** SOD and catalase increased while MDA decreased in a graded fashion (Control < Classical < Modified; Figures 3-5; Table 3). One-way ANOVA indicated strong group effects for each endpoint (F statistics in Table 3); post-hoc contrasts (not shown) favored modified over classical. These patterns agree with prior animal studies and mechanistic work on Triphala's redox modulation [1, 3, 5-8, 10, 11].
- **Metabolic and hepatic indices:** Improvements in LDL and ALT with the modified formulation (Table 3) suggest broader systemic benefits, consistent with earlier observations of lipid and hepatic enzyme modulation under Triphala treatment [1, 6, 8].
- **Anti-angiogenic activity:** CAM vessel density decreased most with the modified formulation (Figure 6; Table 4), coherent with VEGF-A pathway inhibition attributed to chebulinic acid and related constituents [5, 7, 10].
- **Biopharmaceutic/technological advantages:** Enhanced phenolic release at 30 min and a lower angle of repose (Figures 7-8; Table 4) indicate better dissolution and flow, outcomes anticipated from optimized granulation and particle-engineering, as shown in prior formulation studies [12, 13]. The higher total phenolics and chebulinic-acid content in the modified product (Table 1) likely underpin the PD gains, echoing metabolomic and standardization insights for Terminalia/Emblica spp. [2, 7, 9, 14-16].

Overall interpretation: The modified dosage form demonstrated superior pharmacodynamic effect sizes across antioxidant, anti-inflammatory, anti-angiogenic, and *in vivo* biomarker endpoints compared with the classical powder,

plausibly mediated by improved phenolic yield and release characteristics. These results substantiate the a priori hypothesis and support formulation-aware modernization of Triphala for enhanced therapeutic performance [1-16].

Discussion

The comparative pharmacodynamic assessment between the classical Triphala Churna and its modified formulation demonstrates that reformulation significantly enhances multiple biofunctional parameters. These findings corroborate the central hypothesis that improved physicochemical and biopharmaceutical attributes translate into superior pharmacodynamic outcomes [1-3, 6, 8, 10-13].

The antioxidant profile, reflected by higher DPPH radical scavenging and ferric-reducing antioxidant power (FRAP), supports enhanced redox buffering in the modified form. The classical Triphala Churna showed notable activity consistent with historical Ayurvedic pharmacological data [14-16], but the modified granulated form exhibited a greater magnitude of antioxidant response, likely attributable to increased phenolic content and improved solubility [2, 5, 6]. Studies have established that chebulinic acid and gallic acid act synergistically to scavenge reactive oxygen species and inhibit lipid peroxidation [3, 7, 9]. The elevated total phenolic yield (Table 1) reinforces earlier reports that manufacturing processes such as controlled granulation and particle-size reduction increase surface area and extraction efficiency [12, 13].

In anti-inflammatory assays, inhibition of protein denaturation and membrane stabilization improved markedly with the modified formulation. This observation aligns with prior mechanistic analyses showing that Triphala constituents modulate cyclooxygenase and lipoxygenase pathways, contributing to reduced protein denaturation and oxidative inflammation [4, 5, 7, 8]. Enhanced availability of tannins and flavonoids may have potentiated these responses, consistent with findings of Belapurkar *et al*. and Wang *et al*., who linked polyphenolic concentration with higher cellular anti-inflammatory capacity [4, 7].

The *in vivo* biomarker profile substantiates the pharmacological advancement observed *in vitro*. The significant increase in superoxide dismutase (SOD) and catalase levels, along with a concurrent decline in malondialdehyde (MDA), demonstrates the antioxidative reinforcement induced by the modified dosage form [1, 3, 6, 8, 10]. These changes indicate a restoration of enzymatic antioxidant defenses and inhibition of lipid peroxidation, outcomes supported by previous experimental work using Triphala-based formulations in oxidative stress models [3, 6]. Moreover, improvement in serum lipid and hepatic enzyme levels (LDL and ALT) implies potential for broader systemic modulation, as earlier suggested by Lu *et al*. and Sivasankar *et al*. [5, 6].

A notable pharmacodynamic distinction lies in the anti-angiogenic potential, where CAM assay results indicated reduced vessel density in the modified group. This is consistent with chebulinic acid-mediated suppression of VEGF-A-driven angiogenesis reported by Lu *et al*. [5] and supported by contemporary mechanistic studies on polyphenol-induced endothelial regulation [10]. These outcomes collectively highlight the modified formulation's broader therapeutic relevance beyond antioxidation, positioning it as a potential multi-target intervention.

Equally significant are the pharmaceutical and biopharmaceutic improvements. Reduced angle of repose and enhanced phenolic release (Figures 7-8) confirm better flow properties and dissolution kinetics, both critical for dose uniformity and bioavailability [11-13]. This mirrors previous development studies showing that optimized granulation of Triphala reduces variability and improves reproducibility in polyherbal preparations [12, 13]. Improved physicochemical parameters (lower moisture content, higher total phenolics) directly relate to formulation stability and potency retention [9, 14-16].

Collectively, these results affirm that formulation engineering can significantly enhance the pharmacodynamic efficacy of classical Ayurvedic preparations. The modified Triphala Churna offers measurable advantages in antioxidant, anti-inflammatory, anti-angiogenic, and metabolic domains, while retaining the holistic pharmacognostic essence of the traditional blend. By linking pharmaceutic optimization with classical herbal pharmacodynamics, this study bridges traditional Ayurvedic practice and contemporary evidence-based formulation science. These findings advocate for modernization of Ayurvedic dosage forms through validated pharmaceutical processes without compromising the integrity of traditional formulations thus ensuring enhanced efficacy, stability, and global therapeutic acceptance [1-16].

Conclusion

The present experimental investigation conclusively demonstrates that the modified formulation of Triphala Churna possesses superior pharmacodynamic efficacy compared with the classical powdered form across multiple biological domains, including antioxidant, anti-inflammatory, anti-angiogenic, and metabolic modulation activities. The observed enhancements are directly linked to improved physicochemical parameters such as higher phenolic yield, greater chebulinic acid concentration, enhanced dissolution, and better flow properties achieved through controlled granulation and optimized formulation design. These improvements not only strengthen the pharmacological potency but also address long-standing challenges of traditional Ayurvedic powders, such as inconsistent dosing, poor palatability, and suboptimal bioavailability. The experimental outcomes validate the hypothesis that formulation engineering can amplify the inherent therapeutic potential of traditional herbal combinations without altering their classical essence. The results further signify that a refined delivery system enhances the bioefficacy of the phytoconstituents, leading to improved redox homeostasis, stabilization of cellular enzymes, and inhibition of pathological angiogenesis key factors in chronic inflammatory and degenerative diseases.

From a practical perspective, these findings hold immense value for pharmaceutical standardization and clinical translation within Ayurveda and integrative medicine. It is recommended that future production of Triphala-based formulations adopt scientifically standardized granulation, tablet, or capsule technologies to ensure batch-to-batch uniformity and improved therapeutic consistency. Regulatory authorities and manufacturers should consider updating monographs in the Ayurvedic Pharmacopoeia to incorporate validated physicochemical parameters, phenolic profiling, and dissolution benchmarks as quality-control standards. Clinicians and practitioners may prioritize the use

of modified formulations in conditions requiring prolonged administration such as metabolic syndrome, oxidative liver disorders, and inflammatory bowel disease where improved bioavailability ensures better compliance and outcomes. Researchers are encouraged to further investigate pharmacokinetic correlations, dose-response relationships, and long-term safety profiles of modified Triphala preparations using advanced analytical and omics-based approaches. Educational institutions and Ayurveda research centers can integrate formulation science modules into traditional curricula to foster evidence-based modernization of classical drugs. In essence, this study bridges ancient wisdom with modern pharmaceutical innovation, reaffirming that traditional formulations, when re-engineered through contemporary scientific methods, can achieve enhanced clinical performance, global acceptance, and therapeutic reliability without compromising their Ayurvedic authenticity or holistic philosophy.

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