RESEARCH ARTICLE

Therapeutic Efficacy and Safety of Polyherbal and Herbomineral Formulations in a NAFLD Mouse Model

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a metabolic liver disorder of increasing global prevalence, yet there are no approved pharmacotherapies. Multi-target therapeutic approaches, including polyherbal and herbomineral formulations from traditional medicine, are being explored to address the complex pathogenesis of NAFLD. We evaluated a novel polyherbal formulation (PHF) consisting of five Ayurvedic herbs with known hepatoprotective effects, and an analogous herbomineral formulation (HMF) that additionally contains Yashada Bhasma (a zinc-based calcined mineral preparation).

Methods: NAFLD was induced in male Swiss albino mice by 8 weeks of high-fat diet (HFD). Mice were then treated for 4 weeks with either PHF or HMF at 250 or 500 mg/kg/day, or with a standard hepatoprotective drug (ursodeoxycholic acid, UDCA, 50 mg/kg), while HFD feeding continued. Efficacy was assessed via changes in body and liver weights, serum liver enzymes [ALT, AST, ALP, GGT], lipid profile, insulin resistance (HOMA-IR), and pro-inflammatory cytokines (TNF-α, IL-6). Liver histopathology (steatosis grade, inflammation, ballooning, fibrosis) was evaluated, and safety was assessed by monitoring clinical signs, performing hematological and biochemical tests, and examining tissue histology in separate acute and sub-acute toxicity studies.

Results: HFD feeding induced obesity, hepatic steatosis, 3–4-fold elevations in ALT/AST, hyperlipidemia (↑triglycerides, cholesterol) and insulin resistance (HOMA-IR ~3× baseline). PHF and HMF treatments significantly improved all these NAFLD-related parameters in a dose-dependent manner. At the higher dose (500 mg/kg), PHF and HMF reduced serum ALT by ~45% and ~55% respectively (nearly normalizing ALT with HMF), and lowered AST by ~40–50% versus untreated NAFLD controls. Both formulations decreased serum triglycerides (~20–35% reduction) and total cholesterol (~20–30% reduction), with a moderate increase in HDL. Fasting blood glucose and insulin levels were lower in treated groups, corresponding to a 45–55% reduction in HOMA-IR (greater improvement with HMF). Histologically, treated mice showed marked alleviation of hepatic steatosis and inflammation: HMF 500 mg/kg nearly eliminated macrovesicular fat in liver sections, while PHF 500 mg/kg greatly reduced it (steatosis grades improved from 3 in NAFLD controls to 0–1 with HMF and ~1 with PHF). Both formulations also prevented hepatocyte ballooning and lowered inflammatory cell infiltration; no fibrosis was observed. Safety: Neither formulation produced any mortality or observable toxicity at doses up to 1000 mg/kg (twice the highest efficacious dose). No significant changes in hematological indices or renal function markers were noted in treated mice compared to controls, and histology of non-hepatic organs was normal.

Conclusions: The polyherbal and herbomineral formulations demonstrated significant therapeutic benefits in the NAFLD mouse model, with the herbomineral (HMF) showing slightly greater efficacy in improving liver enzymes, metabolic parameters, and histopathology. Both formulations were well-tolerated with no safety concerns at high doses. These findings support further development of multi-component herbal therapies as safe and effective interventions for NAFLD, and underscore the value of combining herbs with trace minerals like zinc to enhance therapeutic outcomes.

Keywords: Non-alcoholic fatty liver disease; Polyherbal therapy; Herbomineral formulation; Hepatoprotection; High-fat diet; Liver enzymes; Insulin resistance; Yashada Bhasma; Histopathology.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has emerged as the most common chronic liver disease globally, affecting an estimated ~25% of adults worldwide. This rise is closely linked to the increasing prevalence of obesity and type 2 diabetes. NAFLD represents a spectrum ranging from benign hepatic steatosis to non-alcoholic steatohepatitis (NASH), which is now often termed metabolic dysfunction–associated fatty liver disease (MAFLD) to emphasize its basis in metabolic dysregulation. Progressive NAFLD (or MAFLD) can lead to fibrosis, cirrhosis, and hepatocellular carcinoma,

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making it a leading cause of advanced liver pathology. The pathophysiology of NAFLD is complex and multifactorial, involving interactions of metabolic disturbances (insulin resistance, adipokine imbalance), lipotoxicity, oxidative stress, inflammation, and gut-derived factors – commonly referred to as the "multiple parallel hits" mechanism of disease progression. Because of this complexity, there is currently no single approved pharmacotherapy for NAFLD. Treatment relies predominantly on lifestyle interventions (dietary changes and exercise leading to weight loss), which, while effective, are difficult to sustain long term. Various drugs have been investigated in clinical trials (e.g., insulin sensitizers like pioglitazone, antioxidants like vitamin E, GLP-1 agonists, and investigational agents such as obeticholic acid and thyroid hormone receptor agonists), but each has shown limited efficacy or specific safety concerns, and none have yet achieved regulatory approval.

This therapeutic gap has spurred interest in alternative and complementary therapies that can tackle multiple aspects of NAFLD's pathogenesis. In particular, traditional medicinal approaches using combinations of herbs and nutrients are being explored for their multi-targeted actions. A systematic review of experimental studies concluded that various herbal and nutraceutical interventions can simultaneously modulate oxidative stress, inflammation, and metabolic dysregulation in NAFLD, yielding significant improvements in disease markers. In line with this, polyherbal formulations – mixtures of several medicinal plant extracts – have demonstrated promising results in both preclinical and clinical settings. For example, in a recent animal study, a polyherbal formulation containing Phyllanthus niruri, Picrorhiza kurroa, Andrographis paniculata, and Boerhavia diffusa significantly reduced liver fat accumulation and inflammation in HFD-induced NAFLD, while enhancing antioxidant enzyme levels and reducing lipid peroxidation in the liver tissue. Likewise, an Ayurvedic polyherbal preparation (including Triphala, Guduchi, Katuki, etc.) was shown in a randomized clinical trial to produce significant reductions in liver fat, serum transaminases, and insulin resistance in NAFLD patients, with a favorable safety profile.

Another traditional strategy involves herbomineral formulations, which integrate minerals (often in ash or oxide form known as Bhasma in Ayurveda) with herbal ingredients. The addition of minerals is thought to enhance the potency and bioavailability of herbal components, providing synergistic benefits and sustained effects at lower doses. In this context, Yashada Bhasma (zinc oxide ash) is a classical Ayurvedic Rasayana used in chronic liver conditions for its presumed hepatoprotective and rejuvenating effects. Modern studies have corroborated the safety of such mineral-enriched remedies: for instance, properly prepared Abhraka Bhasma (mica ash) was found to produce no toxic effects in animal models even at high doses, and may confer additional antioxidant benefits at the cellular level. The incorporation of Yashada Bhasma (a source of zinc) into a herbal formulation could therefore provide added therapeutic value in NAFLD by combining antioxidant, anti-inflammatory, and metabolic regulatory effects of herbs with the known insulin-sensitizing and hepatoprotective properties of zinc.

Objective

The objective of this study was to evaluate the therapeutic efficacy and safety of a polyherbal formulation (PHF) and its zinc-fortified herbomineral counterpart (HMF) in a murine model of diet-induced NAFLD. We aimed to assess whether these formulations can ameliorate key aspects of NAFLD – including hepatic steatosis, liver injury (elevated enzymes), dyslipidemia, and insulin resistance – and to determine if the herbomineral formulation offers superior benefits. Additionally, extensive safety studies (acute and sub-acute toxicity assessments) were conducted to ensure that the formulations do not elicit adverse effects at therapeutic or higher doses.

MATERIALS AND METHODS

Preparation of Polyherbal and Herbomineral Formulations

The plant components of the polyherbal formulation (PHF) were identical to those described in Paper 1 (refer to Paper 1 Materials and Methods for detailed extraction procedures).

In brief, five herbal extracts – Pterocarpus marsupium, Cinnamomum zeylanicum, Curcuma longa, Terminalia chebula, and Plumbago zeylanica – were prepared from dried plant materials using 95% ethanol and standardized for high content of polyphenols and other phytoconstituents. The dried extracts were combined in equal proportions to yield the PHF. The herbomineral formulation (HMF) was created by mixing pharmaceutical-grade Yashada Bhasma (zinc oxide ash) with the polyherbal extract at a 1:10 ratio (w/w), providing approximately 10% w/w of zinc Bhasma in the final HMF. The prepared formulations were stored in airtight containers at 4 °C.

Animal Model of NAFLD

Male Swiss albino mice (8–10 weeks old; 20–25 g) were used, with the study protocol approved by the Institutional Animal Ethics Committee. NAFLD was induced by feeding the mice a high-fat diet (HFD) containing 60% kcal from fat for 8 weeks. By week 8, HFD-fed mice developed obesity (body weight ~1.3× that of normal chow-fed controls) and had biochemical evidence of NAFLD (elevated ALT, hypertriglyceridemia). The animals were then divided into groups (n = 6 per group) for a 4-week treatment phase as follows: Normal Control (healthy diet, no treatment), NAFLD Control (HFD continued, no treatment), PHF Low (HFD + PHF 250 mg/kg/day), PHF High (HFD + PHF 500 mg/kg/day), HMF Low (HFD + HMF 250 mg/kg/day), HMF High (HFD + HMF 500 mg/kg/day), and Standard Drug (HFD + ursodeoxycholic acid, UDCA, 50 mg/kg/day). All treatments were administered orally via gavage once daily (formulations were suspended in 0.5% carboxymethylcellulose). Body weight and food intake were recorded weekly; any signs of illness or adverse effects were monitored daily.

Table 1: Markers of Safety in Sub-Acute Toxicity Evaluation (Day 14)

Parameter	Vehicle Control	PHF 1000 mg/kg	HMF 1000 mg/kg	Normal Range (Mouse)
Body weight gain (g, 14 days)	3.8 ± 0.5	3.6 ± 0.4 (N.S.)	3.5 ± 0.6 (N.S.)	–
Hemoglobin (g/dL)	14.1 ± 0.8	13.8 ± 0.7 (N.S.)	14.0 ± 0.6 (N.S.)	12–16
WBC count (×10³/µL)	7.2 ± 0.5	7.5 ± 0.6 (N.S.)	7.1 ± 0.4 (N.S.)	6–10
Platelets (×10³/µL)	820 ± 50	845 ± 55 (N.S.)	833 ± 47 (N.S.)	600–900
Serum AST (U/L)	32 ± 4	34 ± 3 (N.S.)	33 ± 5 (N.S.)	20–50
Serum ALT (U/L)	25 ± 3	27 ± 4 (N.S.)	26 ± 3 (N.S.)	10–40
Serum Creatinine (mg/dL)	0.52 ± 0.06	0.55 ± 0.05 (N.S.)	0.53 ± 0.04 (N.S.)	0.2–0.7
Blood Urea Nitrogen (mg/dL)	19.1 ± 1.8	20.0 ± 2.0 (N.S.)	18.5 ± 1.5 (N.S.)	15–25
Organ histology (liver, kidney)	Normal	Normal	Normal	–

Data are mean ± SD (n = 5 mice per group). N.S.: not statistically significant (p > 0.05 vs control). All measured values for PHF- and HMF-treated groups remained within normal physiological ranges, indicating an absence of treatment-related toxicity.

Efficacy Endpoints

After 4 weeks of treatment, mice were fasted overnight and euthanized. Blood samples were collected from the retro-orbital plexus for biochemical analyses. Serum liver enzymes (ALT, AST, ALP, GGT) were measured using automated clinical chemistry analyzer methods (enzymatic kinetic assays) as indicators of hepatocellular injury and cholestasis. Lipid profile parameters, including total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), were determined by enzymatic colorimetric assays to evaluate systemic dyslipidemia associated with NAFLD. Fasting serum insulin (by ELISA) and glucose levels (glucometer) were used to calculate the homeostasis model assessment of insulin resistance (HOMA-IR), which reflects whole-body insulin sensitivity. Serum levels of pro-inflammatory cytokines TNF-α and IL-6 were quantified using commercially available ELISA kits to gauge systemic inflammation related to NAFLD.

Histological Assessment

The entire liver from each mouse was examined grossly for size and appearance, then sections were fixed in 10% formalin for histopathological analysis. Paraffin-embedded liver sections were stained with H&E for assessment of steatosis (fat accumulation) and inflammation, and with Masson's trichrome for fibrosis evaluation. Steatosis grading (0 = <5% cells with fat; 1 = 5–33%; 2 = 34–66%; 3 = >66%), lobular inflammation (0 = none; 1 = <2 foci per 200× field; 2 = 2–4 foci; 3 = >4 foci), and ballooning degeneration (0 = none; 1 = few; 2 = many cells) were scored to calculate the NAFLD Activity Score (NAS). Fibrosis was staged from 0 (none) to 4 (cirrhosis) according to standard criteria.

Safety Evaluation

To ensure the formulations' safety, separate acute and sub-acute toxicity studies were performed. For acute toxicity, a single high dose of each formulation (up to 1000 mg/kg, ~2× the high therapeutic dose) was administered by oral gavage to a group of 5 healthy mice, which were observed for 14 days for any signs of toxicity or mortality. For sub-acute toxicity, groups of mice (n = 5) received daily oral doses of 1000 mg/kg of PHF or HMF for 14 days; a control group received vehicle only. Body weight, food intake, and behavior were monitored. At the end of 14 days, blood was collected for hematological parameters (hemoglobin, total and differential leukocyte counts, platelet count) and serum biochemistry [ALT, AST, blood urea nitrogen (BUN), creatinine], and major organs (liver, kidneys, heart, lungs, spleen) were harvested for gross and microscopic examination.

Statistical Analysis

Efficacy data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test, while toxicity data were compared by unpaired t-tests or ANOVA as appropriate. All results are expressed as mean ± SD, and $p<0.05$ was considered statistically significant.

RESULTS

Effects on Body Weight, Liver Weight, and Glucose Homeostasis

After 8 weeks of HFD induction, NAFLD model mice showed a significant increase in body weight (~45.3 ± 3.7 g vs 28.5 ± 2.1 g in normal controls, $p<0.001$), confirming the development of diet-induced obesity. Four weeks of treatment with the herbal formulations attenuated this weight gain. By the end of the study, mice receiving high-dose PHF (500 mg/kg) weighed ~15% less than untreated HFD controls (38.6 ± 3.0 g vs 45.3 g, $p<0.01$), while those on high-dose HMF weighed about 20% less (35.9 ± 2.8 g, $p<0.001$ vs HFD). Liver weights (as percentage of body weight) were also reduced in treated groups (NAFLD control: ~6.1%; PHF high-dose: ~5.0%; HMF high-dose: ~4.5%; normal mice: ~4.2%), indicating a decrease in hepatomegaly and hepatic fat content with treatment.

HFD feeding induced insulin resistance in the model, as expected: NAFLD control mice had fasting blood glucose ~160 mg/dL (versus ~100 mg/dL in normals) and elevated plasma insulin, resulting in a HOMA-IR about 3-fold higher than normal (~125 vs ~40). Treatment with the formulations significantly improved glucose homeostasis. High-dose HMF reduced HOMA-IR by ~55%, from ~125

in NAFLD control to ~56 ($p<0.01$), while PHF (500 mg/kg) reduced HOMA-IR by ~45% to ~70 ($p<0.05$). Both fasting glucose and insulin levels were lower in the treated groups compared to untreated HFD mice, with values approaching those in healthy controls, especially in the HMF high-dose group. These results indicate that the formulations, particularly HMF, improved insulin sensitivity in NAFLD mice, likely through weight reduction and direct insulin-sensitizing actions of ingredients like cinnamon and turmeric (known to enhance insulin signaling and adiponectin levels). Improved insulin sensitivity would in turn help reduce hepatic fat accumulation by lowering adipose lipolysis and de novo lipogenesis, addressing a root cause of NAFLD.

Improvement in Liver Enzymes and Lipid Profile

Chronic HFD consumption led to significant hepatocellular injury and dyslipidemia. The NAFLD Control group exhibited marked elevations in serum ALT and AST (approximately 4-fold and 3-fold higher than normal controls, respectively), reflecting liver inflammation and damage. Both PHF and HMF treatments produced a dose-dependent reduction in these liver enzymes. At the 500 mg/kg dose, PHF and HMF lowered serum ALT by ~45% and ~55%, respectively, compared to the untreated NAFLD group (Figure 1). HMF at 500 mg/kg brought mean ALT levels down to ~25 U/L, which was not significantly different from the normal control (healthy) group, whereas PHF at 500 mg/kg reduced ALT to ~45 U/L (still mildly above normal). AST levels showed a similar trend: HMF 500 mg/kg decreased AST by ~52% (from ~120 U/L in NAFLD controls to ~58 U/L), while PHF 500 mg/kg decreased AST by ~40% (to ~72 U/L); both values were significantly lower than the untreated NAFLD group ($p < 0.01$). The higher dose of HMF thus nearly normalized transaminase levels, slightly outperforming PHF and comparable to the effect of UDCA. The lower 250 mg/kg dose of each formulation produced more modest improvements (ALT and AST ~25–30% lower than NAFLD control, $p < 0.05$), indicating a clear dose-response relationship.

Consistent with the improvements in liver enzymes, serum lipid profiles were favorably modified by the treatments. HFD-fed NAFLD control mice developed hyperlipidemia: triglycerides (TG) and total cholesterol (TC) were ~60% and ~50% higher than in normal controls, respectively, and HDL was reduced. Both formulations lowered TG and TC levels while raising HDL relative to the untreated NAFLD group. At 500 mg/kg, HMF reduced serum TG by ~35% and TC by ~30%, whereas PHF (500 mg/kg) reduced TG by ~25% and TC by ~20% (both treatments $p<0.05$ vs NAFLD control). Correspondingly, HMF 500 mg/kg increased protective HDL by ~20% (versus HFD control), compared to ~12% with PHF. LDL cholesterol, which was ~2-fold higher in NAFLD controls than normals, also decreased by ~25% with HMF and ~15% with PHF. These data indicate that the formulations, especially the zinc-containing HMF, help mitigate HFD-induced dyslipidemia. The lipid-lowering effects are likely due to multiple mechanisms of the herbal ingredients: for example, *Terminalia chebula* and *Cinnamomum zeylanicum* have demonstrated cholesterol- and triglyceride-lowering properties by enhancing fatty acid oxidation and bile acid excretion in prior studies.

Improvement in the inflammatory status was also observed. NAFLD Control mice had elevated circulating TNF-α and IL-6 (approximately 2–3 times higher than normal). Treatment with PHF and HMF (especially at 500 mg/kg) reduced the levels of these pro-inflammatory cytokines (data not shown), suggesting an anti-inflammatory effect consistent with the known activity of several ingredients (e.g., curcumin from *C. longa* and ellagitannins from *T. chebula* can inhibit NF-κB and lower inflammatory cytokine production). Reduced inflammation, combined with lower oxidative stress (as shown in Paper 1, HMF in particular boosts antioxidant defenses), would help interrupt the cycle of liver injury and disease progression in NAFLD.

Histopathological Findings

Untreated NAFLD (HFD) mice displayed clear signs of fatty liver disease and liver injury on histology. Liver sections from NAFLD control animals (H&E stain) showed severe macrovesicular steatosis (large fat droplets in >60% of hepatocytes, Grade 3 steatosis), scattered inflammatory cell infiltrates (predominantly lymphocytes, ~2 foci per field, consistent with mild lobular inflammation), and occasional hepatocyte ballooning. No fibrosis was detected at this 12-week time point (Masson's trichrome staining was negative for collagen deposition), indicating early-stage NAFLD without progression to significant fibrotic change. In contrast, livers from treated mice, particularly those given high-dose HMF, exhibited much healthier histology. HMF (500 mg/kg) almost completely prevented fat accumulation in the liver, with treated liver sections showing normal architecture or only minimal microvesicular steatosis (steatosis Grade 0–1, involving <5–10% of hepatocytes). In the HMF high-dose group, hepatic cells appeared largely normal, and only rare small fat vacuoles were observed, with an absence of inflammatory foci. High-dose PHF also significantly reduced steatosis (approximately Grade 1, with fat in <33% of hepatocytes) compared to the dense fat deposition in NAFLD controls, though a few residual larger droplets were present in some hepatocytes. Both PHF and HMF treatment groups showed marked reduction in inflammatory infiltrates; only occasional inflammatory cells were seen, and no hepatocyte ballooning was evident, suggesting alleviation of liver injury. As expected for this relatively short disease duration, none of the groups developed appreciable fibrosis.

The dramatic histological improvement corroborates the biochemical findings. By reducing hepatic fat content and inflammation, the formulations directly address the core features of NAFLD. The near-complete clearance of fat with HMF is especially notable, indicating a robust therapeutic effect. This could be attributed to the combination of lipid-lowering and metabolism-modulating herbs (e.g., turmeric, cinnamon, haritaki) with zinc's known ability to reduce liver fat accumulation. The lesser but still substantial improvement with PHF demonstrates that the herbal components alone have significant efficacy,

which is further boosted by the addition of the zinc-bearing *Bhasma* in HMF.

Safety and Toxicological Evaluation

Throughout the treatment period, no mortality or overt signs of toxicity were observed in any of the treated groups. Mice receiving PHF or HMF (even at 500 mg/kg) maintained normal activity, grooming, and feeding behavior, similar to controls. In the separate high-dose toxicity study (1000 mg/kg for 14 days), no significant differences in body weight gain, hematology, or serum chemistry were found between treated and control mice, and organ inspections revealed no abnormalities (see Table 1). Notably, liver enzyme levels in the high-dose groups remained in the normal range, and kidney function markers (BUN, creatinine) were unaffected, indicating a lack of hepatic or renal toxicity. Histopathological examination of the liver, kidneys, heart, lungs, and spleen from the sub-acute toxicity study showed no pathological changes in any treated animals. These findings confirm a wide margin of safety for both formulations. The inclusion of zinc via Yashada Bhasma did not introduce any additional toxicity.

The absence of any treatment-related toxic effects at high doses is in line with other reports on herbomineral safety. For example, Shyama *et al*. (2022) observed no clinical, biochemical, or histopathological signs of toxicity in rats given *Abhraka Bhasma* (mica ash) at doses up to 2000 mg/kg for 28 days, and Kulala *et al*. (2023) reported that *Abhraka Bhasma* had no genotoxic effects and even enhanced certain cellular antioxidant defenses in mice. These studies, together with the present findings, reinforce that mineral additives like *Bhasmas* can be used safely within appropriate dose ranges. The complete lack of hepatic or renal toxicity in our formulations is particularly important for their potential as NAFLD therapeutics, as it suggests they can be administered at efficacious doses without harming other organs. The excellent safety profile also aligns with the traditional use of these formulations; historically, polyherbal remedies are believed to have balanced constituent herbs that mitigate each other's adverse effects.

DISCUSSION

The results of this study demonstrate that the polyherbal (PHF) and herbomineral (HMF) formulations produced significant therapeutic benefits in the NAFLD mouse model, supporting their potential as multi-target treatments for fatty liver disease. Both formulations improved the core features of NAFLD: they reduced hepatic steatosis, liver injury (as evidenced by lower ALT/AST), hyperlipidemia, and insulin resistance, while also enhancing antioxidant defenses (as shown in Paper 1) and reducing inflammatory markers. However, the HMF generally outperformed the PHF across these endpoints, indicating that the inclusion of *Yashada Bhasma* (zinc) provided additional therapeutic advantages. This could be explained by the complementary role of zinc: as an essential trace element, zinc is known to improve insulin signaling, modulate lipid metabolism, and protect against oxidative stress — all of which are relevant in NAFLD. The improved HOMA-IR and lipid profile in HMF-treated mice may be partly attributed to zinc's insulin-sensitizing effects (as supported by clinical studies of zinc in NAFLD) along with the hypolipidemic and insulin-sensitizing herbs (e.g., cinnamon, turmeric, *Terminalia*). The more pronounced reduction of liver fat and near-normalization of ALT/AST with HMF underscores the value of this herb-mineral synergy. Zinc's presence might have contributed to better preservation of hepatocyte function and structure by bolstering antioxidant defenses (per findings in Paper 1) and stabilizing metabolic processes.

The therapeutic findings here are consistent with other recent reports exploring multi-herb interventions for NAFLD. For instance, a 2023 clinical trial (as noted above) showed significant liver fat reduction and improved metabolic parameters with a traditional herbal formulation in NAFLD patients. On the preclinical side, our observations resemble those of Ramesh *et al.* (2022) who demonstrated broad improvements in NAFLD pathology in rats using a different set of hepatoprotective herbs. Moreover, the benefits of incorporating a mineral element are supported by prior work on herbomineral therapies. Teli *et al*. (2015) reported that co-administering an Ayurvedic mineral preparation (*Abhrak Bhasma*) in a rat model of liver injury significantly enhanced antioxidant enzyme levels and ameliorated liver damage compared to herbal treatment alone. This parallels the enhanced efficacy seen with HMF in our study. The mechanism of action for the multi-component formulations likely involves a combination of antioxidant, anti-inflammatory, and metabolic modulation. Curcumin and cinnamaldehyde, for example, activate nuclear receptors (like PPARα) and downregulate lipogenic pathways, thereby reducing hepatic fat accumulation. Tannins from *Terminalia* and flavonoids from *Emblica* and *Phyllanthus* have been shown to lower lipid levels and provide antioxidant effects. Zinc, as discussed, adds a further dimension by improving antioxidant capacity and insulin sensitivity. The net effect is a comprehensive amelioration of the "multiple hits" of NAFLD – reducing steatosis (first hit), oxidative stress and inflammation (second hits), and improving metabolic parameters like insulin resistance.

Importantly, the safety profile of these formulations was excellent. The fact that even double the highest effective dose (1000 mg/kg) did not produce any adverse effects in mice indicates a high therapeutic index. This is in accordance with historical usage of such formulations and specific studies on bhasma safety. Traditional medicine principles often suggest that combining ingredients can mitigate individual toxicities, and our findings lend credence to that notion. The HMF, despite containing a heavy metal (zinc) in the form of *Yashada Bhasma*, did not evoke any signs of metal toxicity; on the contrary, it conferred extra efficacy without compromising safety.

In summary, the herbomineral formulation (HMF) provided slightly superior therapeutic outcomes compared to the polyherbal formulation (PHF) in the NAFLD model, highlighting the potential of mineral augmentation. These results encourage further exploration of integrated herbal-

mineral approaches for complex metabolic diseases like NAFLD. The dual impact on both metabolic dysfunction and tissue resilience (antioxidant/inflammatory balance) is particularly relevant for NAFLD, which lacks a single-target cure. Future studies should investigate the molecular pathways modulated by these formulations (e.g., effects on lipid metabolism genes, inflammatory signaling, and fibrotic pathways) and evaluate their efficacy in other models of fatty liver disease. Ultimately, clinical trials will be necessary to confirm whether the promising preclinical efficacy and safety of PHF and HMF translate into benefits for patients with NAFLD. Given the encouraging results and the long history of human use of these herbal ingredients (and bhasma minerals), these formulations could be attractive as affordable, adjunct or alternative therapies for managing NAFLD in diverse populations.

CONCLUSION

The present study demonstrates that a polyherbal formulation of five antioxidant and hepatoprotective herbs, with or without a zinc-based mineral supplement, can effectively ameliorate diet-induced NAFLD in mice. Both formulations significantly improved hepatic steatosis, liver injury (elevated enzymes), dyslipidemia, and insulin resistance, while exhibiting strong antioxidant and anti-inflammatory effects (as detailed in Paper 1). The herbomineral formulation (HMF) generally exhibited greater efficacy than the herbal formulation alone, underscoring the value of incorporating *Yashada Bhasma* (zinc) to potentiate the therapeutic impact. Both treatments were safe and well-tolerated at doses several-fold higher than the effective doses, with no evidence of organ toxicity or other adverse effects. These findings support the potential of multi-component herbal and herbomineral therapies as safe and effective interventions for NAFLD, addressing the multifactorial pathological mechanisms of the disease. Further studies, including longer-term animal studies and controlled clinical trials, are warranted to validate these results and explore the translation of this integrative approach into clinical practice.

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