

Antioxidant Evaluation of a Polyherbal and Herbomineral Formulation for Nafld

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ABSTRACT

Background: Oxidative stress is a key driver in the progression of non-alcoholic fatty liver disease (NAFLD) to steatohepatitis and fibrosis. Multi-herb (polyherbal) formulations and herbomineral preparations (herbal mixtures combined with minerals) are traditional therapeutic strategies to combat chronic liver diseases, potentially targeting multiple pathological factors. In this study, a polyherbal formulation (PHF) composed of five antioxidant-rich medicinal plant extracts, and a corresponding herbomineral formulation (HMF) (the same five herbs fortified with *Yashada Bhasma* – calcined zinc ash) were evaluated for their antioxidant potential in the context of NAFLD.

Methods: Antioxidant capacity was assessed *in vitro* through free radical scavenging assays (DPPH, ABTS, hydroxyl radical, nitric oxide) and by measuring activities of key antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), etc.] and levels of non-enzymatic antioxidants [reduced glutathione (GSH), vitamins C and E] in the formulations. *In vivo* antioxidant effects were tested using a high-fat diet (HFD) induced NAFLD mouse model. Mice were treated with PHF or HMF (250 mg/kg or 500 mg/kg daily, orally) for 4 weeks, and hepatic antioxidant parameters (SOD, CAT, GPx, GSH) and lipid peroxidation (malondialdehyde, MDA) were measured in liver tissue.

Results: Both PHF and HMF demonstrated strong antioxidant activity. In free radical assays, HMF showed greater DPPH and ABTS radical scavenging ($IC_{50} \approx 50 \mu\text{g/mL}$ for DPPH) compared to PHF ($IC_{50} \approx 63 \mu\text{g/mL}$) and even to ascorbic acid ($IC_{50} \approx 66 \mu\text{g/mL}$). Enzymatic assays showed significantly higher activities of SOD, CAT, GPx, and glutathione-S-transferase in HMF than PHF ($p < 0.05$ for all). HMF also markedly increased GSH levels (~8-fold higher than PHF) and vitamin E content relative to PHF. In HFD-fed NAFLD mice, both PHF and HMF restored hepatic antioxidant enzymes and GSH levels toward normal, while reducing MDA (lipid peroxidation marker) by ~30% versus untreated NAFLD controls. Notably, HMF produced greater improvements than PHF in nearly all parameters (e.g., hepatic SOD, CAT, and GPx were ~10–30% higher with HMF than PHF at the same dose, and MDA reduction was greater with HMF).

Conclusion: The polyherbal and herbomineral formulations exhibit potent antioxidant effects *in vitro* and *in vivo*. The inclusion of *Yashada Bhasma* (zinc) in the herbomineral formulation significantly enhanced its antioxidant efficacy, likely by augmenting both enzymatic and non-enzymatic antioxidant defenses. These results suggest that multi-component herbal formulations, especially when fortified with trace minerals such as zinc, can effectively counteract oxidative stress in NAFLD. This antioxidant strategy may help prevent progression of NAFLD and merits further investigation.

Keywords: Non-alcoholic fatty liver disease; Oxidative stress; Antioxidant enzymes; Polyherbal formulation; Herbomineral formulation; *Yashada Bhasma*; *Pterocarpus marsupium*; *Curcuma longa*.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a prevalent chronic liver condition characterized by excessive fat accumulation in the liver unrelated to alcohol consumption. NAFLD affects roughly 25–30% of the global population, and its prevalence has been rising worldwide in parallel with obesity and type 2 diabetes. It is closely associated with metabolic syndrome features such as central obesity and insulin resistance. In a significant subset of patients, simple steatosis can progress to the more severe metabolic dysfunction-associated steatohepatitis (MASH), accompanied by hepatic inflammation and injury, potentially leading to fibrosis, cirrhosis, and hepatocellular carcinoma. The pathogenesis of NAFLD is multifactorial

and is often described by the “multiple-hit” hypothesis, wherein factors like insulin resistance, excess fatty acids, gut-derived endotoxins, and oxidative stress collectively drive disease progression. Among these, oxidative stress – an imbalance between pro-oxidant species and antioxidant defenses – is recognized as a key contributor to hepatocellular injury and fibrogenesis in NAFLD. Reactive oxygen species and lipid peroxidation byproducts can trigger cellular damage and inflammatory pathways, thereby accelerating the advancement from benign steatosis to steatohepatitis. Consequently, enhancing antioxidant defenses has been proposed as a promising therapeutic strategy for preventing or slowing NAFLD progression.

Current standard care for NAFLD centers on lifestyle modification – primarily weight loss through diet and exercise – as no pharmacotherapy is yet universally approved for this condition. Several drugs have been investigated (e.g., *pioglitazone*, *vitamin E*, GLP-1 agonists, SGLT2 inhibitors), but each has shown only modest benefits or specific applicability, often accompanied by safety or tolerability issues. For instance, high-dose vitamin E supplementation in non-diabetic NAFLD (NASH) patients can improve liver histology (reducing steatosis and inflammation), but long-term use raises safety concerns and is not advised for certain groups (e.g., diabetic patients). In the absence of a single effective medication, there is growing interest in multi-target therapeutic approaches to address the complex pathology of NAFLD. Polyherbal formulations, which combine multiple medicinal plants, have a long history in traditional medicine and are thought to act via synergistic mechanisms on various facets of disease (antioxidant, anti-inflammatory, metabolic regulation) to produce greater efficacy than single compounds. Indeed, numerous studies on phytotherapy for NAFLD support this multi-faceted approach. Indian Ayurvedic medicine describes many herbs with hepatoprotective, antioxidant, and anti-inflammatory properties, such as *Emblica officinalis* (amla), *Terminalia chebula* (haritaki), *Tinospora cordifolia* (guduchi), *Picrorhiza kurroa* (katuki), *Phyllanthus niruri* (bhumi amla), *Curcuma longa* (turmeric), *Cinnamomum zeylanicum* (cinnamon), and *Plumbago zeylanica* (chitrak). These have traditionally been used to treat liver disorders and metabolic imbalances. For example, a randomized controlled trial demonstrated that cinnamon supplementation significantly improved serum liver enzymes, triglycerides, and insulin sensitivity in NAFLD patients. Combinations of such herbs have also shown additive benefits in experimental models of fatty liver disease. Ramesh *et al.* (2022) reported that a polyherbal blend of *Phyllanthus*, *Picrorhiza*, *Andrographis*, and *Boerhavia* significantly improved hepatic antioxidant enzyme levels (\uparrow SOD, \uparrow CAT) and reduced malondialdehyde (MDA) and inflammatory cytokines in HFD-fed rats, along with a marked reduction in liver fat and transaminases. Similarly, Sharma *et al.* (2023) found that an Ayurvedic polyherbal formulation containing *Triphala*, *Katuki*, and *Guduchi* led to decreased liver fat, lower ALT and AST, improved insulin resistance, and no significant adverse effects in NAFLD patients over 3 months of treatment. These studies underscore the potential of multi-herb therapies to modulate oxidative stress and metabolic dysfunction in NAFLD.

In addition to herbal components, trace minerals such as zinc also have hepatoprotective potential. Zinc is a cofactor for numerous antioxidant enzymes (e.g., Cu/Zn-SOD) and has been shown to improve liver enzyme levels and reduce hepatic steatosis in both NAFLD patients and animal models. Ayurvedic practice employs *Yashada Bhasma* (a calcined zinc preparation) as a rejuvenating Rasayana for liver ailments. Importantly, preclinical studies indicate that *Yashada Bhasma* is well-tolerated at therapeutic doses and can enhance antioxidant defenses without organ toxicity.

These insights suggest that combining antioxidant-rich herbs with a biometal like zinc could provide a multi-modal approach to ameliorate oxidative stress and related metabolic disturbances in NAFLD.

Objectives: Based on this rationale, the present study aimed to formulate a polyherbal and a herbomineral preparation using selected antioxidant and hepatoprotective herbs (with *Yashada Bhasma* as the mineral additive in the latter), and to evaluate and compare their *in vitro* and *in vivo* antioxidant activities. We hypothesized that the inclusion of *Yashada Bhasma* in the herbomineral formulation would enhance the overall antioxidant efficacy relative to the polyherbal formulation alone. The work focused on two aspects: (1) *In vitro* free radical scavenging capacity and effects on antioxidant enzymes of the formulations; (2) *In vivo* effects on hepatic oxidative stress markers and antioxidant defenses in a mouse model of diet-induced NAFLD.

MATERIALS AND METHODS

Formulation Composition and Preparation: Five medicinal plants with documented antioxidant and hepatoprotective properties were selected for the polyherbal formulation (PHF), based on literature and traditional use. These were: *Pterocarpus marsupium* Roxb. (Indian Kino tree, heartwood), *Cinnamomum zeylanicum* (Ceylon cinnamon, bark), *Curcuma longa* (turmeric, rhizome), *Terminalia chebula* (haritaki, fruit pericarp), and *Plumbago zeylanica* (chitrak, root). Raw materials were authenticated and dried. Coarse powders of each plant part were prepared and extracted individually by Soxhlet extraction with 95% ethanol as solvent (10:1 v/w solvent-to-material ratio). The extracts were filtered and concentrated under reduced pressure below 60 °C to obtain semi-solid masses, which were then dried to yield powdered extracts. Equal quantities (by weight) of each of the five extracts were homogeneously blended to formulate the polyherbal extract (PHF).

For the herbomineral formulation (HMF), the above polyherbal blend was fortified with *Yashada Bhasma* (zinc oxide ash). *Yashada Bhasma* was obtained from an established Ayurvedic pharmacy, prepared via classical incineration and purification processes. The zinc bhasma was mixed with the combined herbal extract in a 1:10 ratio (mineral:herbal, w/w) to produce the herbomineral extract (HMF). Both PHF and HMF were stored in airtight containers at 4 °C until use to preserve phytochemical stability.

In Vitro Phytochemical Screening: The PHF and HMF were subjected to preliminary phytochemical analyses to identify major classes of bioactive constituents. Standard qualitative tests (e.g., ferric chloride test for phenolics/tannins, Shinoda test for flavonoids, Dragendorff's reagent for alkaloids, foam test for saponins) were performed as per standard methods. Total phenolic content was quantified by the Folin–Ciocalteu method (expressed as mg gallic acid equivalents per gram extract), and total flavonoid content by the aluminum chloride colorimetric method (mg quercetin equivalents per gram).

Key antioxidant constituents expected in the formulations included polyphenols such as curcuminoids (from *C. longa*), tannins (gallic and ellagic acids) from *T. chebula*, stilbenoids (e.g., pterostilbene) from *P. marsupium*, and naphthoquinones (e.g., plumbagin) from *P. zeylanica*. The presence of these and other phytochemicals was confirmed in both PHF and HMF extracts, with comparable or greater total phenolic and flavonoid contents in HMF (data not shown).

In Vitro Antioxidant Assays: A battery of antioxidant assays was used to characterize the free radical scavenging ability of PHF and HMF. The DPPH radical scavenging assay was performed by incubating serial concentrations of each extract (10–100 µg/mL) with 0.1 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) solution in methanol. After 30 min in the dark, the decrease in absorbance was measured at 517 nm, and % radical inhibition was calculated relative to a methanol control. The concentration of extract required to scavenge 50% of DPPH (IC₅₀) was determined from inhibition curves. The ABTS radical cation decolorization assay was conducted using 7 mM ABTS and 2.45 mM potassium persulfate to generate the ABTS radical, which was then reacted with the extracts (at various concentrations) and the reduction in absorbance at 734 nm measured. Hydroxyl radical scavenging activity was evaluated by the deoxyribose degradation method (measuring malondialdehyde-thiobarbituric acid adduct at 532 nm) in the presence of iron-EDTA, ascorbate, and hydrogen peroxide. **Nitric oxide (NO) scavenging was assessed using sodium nitroprusside in phosphate buffered saline (incubation at 25 °C for 2 h) and measuring nitrite formation via Griess reagent at 546 nm. In all assays, a well-known antioxidant, ascorbic acid (vitamin C), was included as a reference standard.**

Antioxidant enzyme assays: To probe the effects on antioxidant defense mechanisms, the activity of key antioxidant enzymes in the presence of each formulation was measured *in vitro*. Enzyme preparation was obtained by incubating the herbal extracts with known substrates or enzyme sources. Superoxide dismutase (SOD) activity was determined by the nitroblue tetrazolium (NBT) reduction method, wherein one unit of SOD activity is defined as the amount of sample protein required to inhibit the NBT reduction rate by 50% (monitored at 560 nm). Catalase (CAT) activity was measured based on the decomposition rate of H₂O₂ at 240 nm. Glutathione peroxidase (GPx) activity was assayed via a coupled reaction with glutathione reductase, monitoring NADPH oxidation at 340 nm. Glutathione-S-transferase (GST) activity was determined by the formation of a glutathione-1-chloro-2,4-dinitrobenzene conjugate (absorbance at 340 nm). In addition, levels of non-enzymatic antioxidants in each extract were quantified: reduced glutathione (GSH) using Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid) at 412 nm, vitamin C by the DCPIP dye method, and vitamin E (α-tocopherol) by colorimetry using ferric chloride and α,α'-dipyridyl reagents. All assays were

performed in triplicate for each sample concentration. Results were expressed as mean ± standard deviation (SD).

In Vivo NAFLD Induction and Treatment: Animals and diet: Male Swiss albino mice (8 weeks old, ~25 g) were obtained and acclimated for one week with standard rodent chow and water *ad libitum*. NAFLD was induced by feeding a high-fat diet (HFD) providing 60% of calories from fat for 8 weeks. By the end of this induction period, HFD-fed mice developed obesity (30–40% greater body weight than controls) and liver steatosis (confirmed by elevated plasma ALT and histology showing macrovesicular fatty change). The mice were then randomized into groups (n = 6 per group) for the treatment phase as follows: Normal control (healthy diet, no treatment), NAFLD control (HFD continued, no treatment), PHF 250 (HFD + PHF 250 mg/kg/day), PHF 500 (HFD + PHF 500 mg/kg/day), HMF 250 (HFD + HMF 250 mg/kg/day), HMF 500 (HFD + HMF 500 mg/kg/day), and a Standard treatment group (HFD + ursodeoxycholic acid, UDCA, 50 mg/kg/day). Treatments were administered once daily by oral gavage for 4 weeks. The high dose (500 mg/kg) was chosen to assess efficacy, while the low dose (250 mg/kg) represents a typical therapeutic herbal dose. Body weights and general health were monitored weekly.

After 4 weeks of treatment, mice were euthanized under anesthesia. Blood was collected for serum biochemical assays and liver tissues were harvested for analysis. Serum liver function enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and γ-glutamyl transferase (GGT)] were measured using standard enzymatic kits to evaluate hepatic injury. Serum lipid profile (triglycerides, total cholesterol, low-density and high-density lipoproteins – LDL-C, HDL-C) was determined by colorimetric assays to gauge systemic metabolic effects. Fasting blood glucose and insulin levels were measured (glucometer and ELISA, respectively) after an overnight fast; insulin resistance was estimated by the homeostatic model assessment (HOMA-IR). Circulating inflammatory cytokines [tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6)] were quantified using ELISA kits to assess systemic inflammation.

Hepatic antioxidant and oxidative stress markers: Portions of liver were immediately homogenized in ice-cold phosphate buffer (pH 7.0). Liver homogenates were analyzed for antioxidant enzymes (SOD, CAT, GPx, GST, glutathione reductase, glucose-6-phosphate dehydrogenase) using the same spectrophotometric methods described above for the *in vitro* assays, with activities normalized to liver protein content. Levels of reduced glutathione (GSH) in liver tissue were measured via the DTNB assay. Lipid peroxidation in the liver was determined by the thiobarbituric acid reactive substances (TBARS) assay, which quantifies malondialdehyde (MDA) as a measure of oxidative damage to lipids (absorbance of the MDA-TBA adduct at ~532 nm).

Histopathological Analysis: Representative liver samples from each group were fixed in 10% neutral buffered formalin, processed, and embedded in paraffin. Thin

sections (5 µm) were cut and stained with hematoxylin and eosin (H&E). Blinded histological evaluation was performed to assess the degree of steatosis (graded 0–3 based on percentage of hepatocytes with fat vacuoles), lobular inflammation (score 0–3), and hepatocellular ballooning (0–2), which together contribute to the NAFLD Activity Score (NAS). Fibrosis was assessed on Masson’s trichrome–stained sections, staged 0–4 according to standard NASH Clinical Research Network criteria.

Statistical Analysis: Data were expressed as mean ± SD. Group comparisons were made using one-way ANOVA followed by appropriate post-hoc tests (Tukey’s or Dunnett’s) for multiple comparisons. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical Composition of PHF and HMF

Preliminary phytochemical screening confirmed that both the polyherbal (PHF) and herbomineral (HMF) formulations are rich in metabolites known for antioxidant activity. Qualitative tests indicated the presence of phenolics, flavonoids, tannins, saponins, alkaloids, terpenoids, and glycosides in the extracts. Quantitative analysis revealed substantial total phenolic content (~32 mg gallic acid equivalents/g in PHF) and notable flavonoid content (~12 mg quercetin equivalents/g). These phytochemicals – including curcuminoids (from *C. longa*), tannins (from *T. chebula*), stilbenoids such as pterostilbene (from *P. marsupium*), and naphthoquinones such as plumbagin (from *P. zeylanica*) – are well-known for their radical scavenging and metal-chelating properties, thus providing a biochemical basis for the strong antioxidant effects observed. The herbomineral formulation HMF contains all these herbal constituents and, in addition, supplies zinc in a bioaccessible form. The presence of *Yashada Bhasma* (containing zinc) may further induce antioxidant response pathways (e.g., upregulating metallothioneins or zinc-dependent enzymes), potentially enhancing the overall antioxidant capacity of HMF compared to PHF.

In Vitro Free Radical Scavenging Activity

Both PHF and HMF demonstrated potent *in vitro* free radical scavenging in a concentration-dependent manner, with HMF consistently more active than PHF across all assays. In the DPPH assay, HMF showed an IC₅₀ of approximately 50 µg/mL, compared to ~63 µg/mL for PHF and ~66 µg/mL for ascorbic acid (vitamin C). This indicates that HMF achieved 50% DPPH neutralization at a lower concentration than PHF or even the standard antioxidant

(ascorbic acid), reflecting greater free-radical quenching potency by HMF. In agreement, the ABTS radical cation decolorization assay revealed that HMF at 100 µg/mL caused >90% reduction of the ABTS•⁺ absorbance, whereas PHF achieved ~80% inhibition at the same concentration. Both extracts also effectively scavenged hydroxyl radicals and nitric oxide, reducing levels of these reactive species *in vitro*; notably, HMF’s scavenging activity was approximately 10–20% higher than PHF at equivalent concentrations ($p < 0.01$). *Figure 1 illustrates the DPPH scavenging activities of the formulations.* These data clearly show the superior free radical scavenging capacity of the herbomineral formulation. The enhanced performance of HMF suggests a synergistic interaction between the herbal constituents and the added zinc. Zinc may contribute to radical quenching and bolster the redox cycling of herbal antioxidants, thereby potentiating the overall radical-scavenging effect of the HMF.

Enzymatic and Non-Enzymatic Antioxidant Defense Parameters

The two formulations also activated antioxidant defense systems to different extents. Table 1 summarizes key antioxidant enzyme activities and non-enzymatic antioxidant levels measured in the presence of PHF vs HMF *in vitro*. The herbomineral formulation showed significantly higher activities of SOD, CAT, GPx, and GST in comparison to the polyherbal extract (HMF values were 1.5- to 5-fold greater than PHF for these enzymes). For example, HMF demonstrated an SOD activity of ~8.4 U/mg protein, nearly 3 times that of PHF (~3.0 U/mg). Catalase activity in HMF was 17.0 U/mg vs 8.0 U/mg in PHF. Similarly, HMF doubled the GPx activity and increased GST activity more than tenfold relative to PHF. Correspondingly, non-enzymatic antioxidant levels were markedly elevated in HMF: the herbomineral extract’s GSH content (~219 µmol/mg) was about 8-fold higher than that of PHF (~26 µmol/mg). HMF also contained a higher concentration of vitamin E (α-tocopherol) than PHF. Vitamin C content was comparable between the two formulations. The much greater GSH level in HMF suggests that the presence of zinc may have helped preserve or regenerate intracellular glutathione (by supporting glutathione reductase activity and metallothionein induction). The significantly enhanced enzyme activities in HMF (particularly SOD and GST) are likely due to a combination of higher baseline antioxidant enzyme co-factors in the herbal blend and the ability of zinc to induce expression or stabilize these enzymes.

Table 1: Enzymatic and Non-Enzymatic Antioxidant Parameters of PHF vs HMF (In Vitro)

Antioxidant Parameter	PHF (Extract)	HMF (Extract)	Significance
Superoxide Dismutase (SOD)	3.0 ± 0.2 U/mg protein	8.4 ± 0.2 U/mg protein	<0.01 vs PHF
Catalase (CAT)	8.0 ± 0.1 U/mg protein	17.0 ± 0.1 U/mg protein	<0.01 vs PHF
Glutathione Peroxidase (GPx)	0.8 ± 0.0 U/mg protein	1.8 ± 0.2 U/mg protein	<0.05 vs PHF
Glutathione S-Transferase	0.3 ± 0.1 U/mg protein	4.6 ± 0.2 U/mg protein	<0.01 vs PHF
G6PDH (NADPH gen.)	0.6 ± 0.1 U/mg protein	0.7 ± 0.1 U/mg protein	N.S. (p>0.05)
Reduced Glutathione (GSH)	26.1 ± 1.2 µmol/mg prot.	219.0 ± 1.2 µmol/mg prot.	<0.001 vs PHF
Vitamin C (Ascorbate)	41.3 ± 0.1 mg/g extract	38.0 ± 0.2 mg/g extract	N.S. (p>0.05)

Vitamin E (α-Tocopherol)	~140 mg/100 g extract	~180 mg/100 g extract	–
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Data are mean \pm SD ($n=3$ determinations). N.S.: not significant. Higher activities or levels in HMF vs PHF were statistically significant for all parameters listed as $p<0.05$ (or lower) in the Significance column.

In summary, the herbomineral formulation (HMF) elicited significantly stronger effects on antioxidant defenses than the polyherbal formulation (PHF) in vitro. The presence of zinc likely contributed to this enhancement by serving as a cofactor for antioxidant enzymes (notably Cu/Zn-SOD) and possibly upregulating antioxidant pathways (e.g., the Nrf2-mediated response). The results support the idea that integrating a mineral component in a herbal formulation can amplify both direct radical-scavenging activity and the activation of endogenous antioxidant mechanisms.

In Vivo Antioxidant Efficacy in NAFLD Mice

In HFD-fed mice, chronic administration of PHF and HMF for 4 weeks led to significant improvements in hepatic antioxidant status and reductions in oxidative damage, with a clear dose-dependent effect. High-fat diet feeding alone (NAFLD Control) caused marked oxidative stress in the liver, evidenced by depressed antioxidant enzyme activities and elevated MDA levels compared to normal healthy mice. Both PHF and HMF reversed these detrimental changes. Hepatic SOD activity, which was reduced by ~50% in NAFLD control mice versus healthy controls, was restored toward normal by treatment. PHF at 500 mg/kg increased SOD activity by ~45% (vs untreated NAFLD), while HMF at 500 mg/kg raised SOD by ~60%, nearly normalizing the activity. A similar pattern was observed for catalase and GPx: HFD feeding reduced CAT and GPx activities, whereas both PHF and HMF treatments significantly increased these enzymes ($p<0.05$ vs NAFLD control), with HMF yielding higher enzyme levels than PHF at the same dose. Hepatic GSH (a critical non-enzymatic antioxidant) was depleted in NAFLD control mice to ~50% of normal values; PHF and HMF significantly elevated GSH, and HMF 500 mg/kg fully restored liver GSH to the level of healthy controls.

Concomitantly, the accumulation of lipid peroxidation byproducts in the liver was mitigated. Untreated NAFLD mice showed high MDA (TBARS) levels — approximately double that of normal mice, indicating substantial oxidative injury to liver lipids. Both PHF and HMF significantly reduced hepatic MDA concentrations. HMF at 500 mg/kg lowered MDA by about 33% relative to NAFLD controls (bringing MDA close to normal levels), whereas PHF at 500 mg/kg reduced MDA by ~25%. These findings reflect improved redox balance and protection against fat-induced oxidative damage in the liver, especially with the herbomineral formulation.

Mechanistic considerations: The greater efficacy of HMF in vivo suggests that adding *Yashada Bhasma* (zinc) provided an “antioxidant advantage” by bolstering both enzymatic and non-enzymatic defenses. Zinc’s known roles include stabilizing the structure of antioxidant enzymes

(like SOD1) and inducing the expression of metallothioneins, which can sequester reactive metals and scavenge radicals. The enhanced GSH levels seen with HMF may be linked to zinc’s ability to preserve glutathione by upregulating glutathione recycling and synthesis. By mitigating oxidative stress, the formulations — particularly HMF — target a central mechanism of NAFLD pathogenesis. Lower oxidative stress in the liver can translate to reduced activation of inflammation and stellate cell-mediated fibrosis, potentially slowing the progression of NAFLD to more advanced liver disease. This multi-faceted antioxidant action is a key advantage of using complex herbal mixtures with added micronutrients in managing chronic liver conditions.

Our findings align with previous research on herbomineral therapies for liver protection. For instance, Teli *et al.* (2015) demonstrated that an *Abhrak Bhasma* (mica-based mineral formulation) significantly increased hepatic SOD and CAT activities and reduced MDA levels in rats with CCl₄-induced liver toxicity, paralleling the improvements in antioxidant markers observed with HMF in the present study. Such evidence supports the traditional rationale of combining herbs with minerals to potentiate therapeutic effects. The results from this study provide a scientific basis for the Ayurvedic concept of Rasayana synergy, wherein a mineral additive can amplify the efficacy of herbal medicines.

CONCLUSION

The antioxidant evaluation of the polyherbal and herbomineral formulations highlights their potential in counteracting oxidative stress, a major contributor to NAFLD progression. Both PHF and HMF exhibited robust free radical scavenging activities and enhanced antioxidant defenses, with HMF demonstrating consistently greater effects than PHF. The addition of *Yashada Bhasma* (zinc) to the polyherbal base significantly improved its performance, likely through synergistic mechanisms that boost both direct radical scavenging and upregulation of endogenous antioxidant systems. These findings suggest that multi-component herbal therapies, especially herbomineral formulations, may serve as effective adjuncts in the management of NAFLD by targeting oxidative stress. Future studies should investigate the precise molecular pathways through which these formulations exert their antioxidant effects (such as Nrf2 pathway activation and mitochondrial protection) and evaluate their impact on steatosis, inflammation, and fibrosis in NAFLD. The promising results warrant further preclinical and clinical development of such multi-modal antioxidant therapy for NAFLD.

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