

Anti-Inflammatory and Hepatoprotective Properties of Herbal Extracts in Non-Alcoholic Fatty Liver Disease Models

Angesh Kumar¹, Vipin Kumar Garg^{*2}, Dushyant Kumar Mishra³, Manoj Kumar⁴

¹Associate Professor, Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology (MIET), NH-58, Baghpat bypass crossing road, Meerut-250005(UP) India

²Professor, Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology (MIET), Meerut

³HoD, Pharmacy Department, United College of Engineering and Research Pharmacy, Greater Noida

⁴Assistant Professor, Kalka Institute for Research and Advanced Studies Partapur bypass, Meerut.

***Corresponding author: Vipin Kumar Garg; vipin.garg@miet.ac.in**

KEYWORDS

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) represents a widespread health concern worldwide, marked by the accumulation of fat in the liver and associated inflammation, while facing a scarcity of effective pharmacological treatments. This research explores the liver-protective benefits of curcumin (*Curcuma longa*) and bergamot (*Citrus bergamia*), emphasising their roles in reducing inflammation, combating oxidative stress, and modulating lipid levels. In vitro investigations involving HepG2 cells and in vivo research utilising HFD-induced NAFLD rat models revealed that both extracts separately enhanced biochemical indicators, cytokine concentrations, and histological structure. The combination therapy produced the most remarkable outcomes, showcasing synergistic decreases in ALT, AST, triglycerides, and markers of oxidative stress, while also enhancing NAFLD Activity Scores. Curcumin, through its mechanisms, suppressed NF- κ B signalling, whereas bergamot bolstered antioxidant defences. The results indicate the promising possibility of integrating curcumin and bergamot as supportive treatments for NAFLD. Additional clinical investigations are essential to confirm these encouraging results and to establish standardised formulations for broader application.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) represents a significant worldwide health issue, impacting around 25% of the global population and becoming the primary contributor to chronic liver conditions (Le et al., 2023). NAFLD includes a range of liver disorders, extending from uncomplicated hepatic steatosis to more advanced stages like non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and eventually hepatocellular carcinoma. The development of NAFLD is influenced by various factors, including genetic susceptibility, metabolic imbalances, oxidative damage, and persistent inflammation. At the heart of this advancement lies the buildup of triglycerides within hepatocytes, which initiates oxidative harm and a series of inflammatory reactions, ultimately compromising both the function and structure of the liver (Guo et al., 2022). At present, the approach to managing NAFLD emphasises changes in lifestyle, such as modifications to diet and enhancements in physical activity, given that there are no pharmacological treatments approved by the FDA that specifically address this condition. The utilisation of medications like pioglitazone and GLP-1 receptor agonists for purposes not officially approved has shown restricted effectiveness in tackling the root causes of inflammation and oxidative stress, raising issues regarding potential side effects and adherence among patients (Younossi et al., 2023). With the increasing incidence of NAFLD, especially amid the worldwide obesity crisis, there is a pressing demand for alternative or supplementary treatment strategies that are both safe and effective, as well as easily accessible. Herbal medicine has surfaced as a hopeful path for the treatment of NAFLD, thanks to its abundant supply of bioactive compounds recognised for their liver-protective and anti-inflammatory effects. Herbal extracts like *Curcuma longa* (curcumin), *Citrus bergamia* (bergamot), and *Artemisia annua* are especially noteworthy due to their capacity to influence various pathways associated with the advancement of NAFLD. Curcumin, the key ingredient found in turmeric, demonstrates strong anti-inflammatory and antioxidant properties by blocking nuclear factor kappa B (NF- κ B) and stimulating the AMP-activated protein kinase (AMPK) pathway, which oversees lipid metabolism and mitigates oxidative stress (Xiao et al., 2013). In a similar vein, bergamot, a citrus fruit abundant in flavonoids like naringin and hesperidin,

has demonstrated the ability to enhance lipid profiles, diminish hepatic fat buildup, and mitigate oxidative harm (Musolino et al., 2020). *Artemisia annua*, well-known for its artemisinin composition, has shown promise in influencing pro-inflammatory cytokines and enhancing liver function indicators in preclinical research (Han et al., 2020). Although there is an increasing amount of evidence that highlights the liver-protective capabilities of these herbal extracts, considerable obstacles still persist. The inconsistency in extract composition, absence of standardisation, and the scarcity of extensive clinical trials have obstructed their incorporation into conventional medical practice (Liang et al., 2021). Moreover, the collaborative impacts of integrating various herbal extracts are still largely unexamined, even though there is significant potential to improve therapeutic effectiveness via multi-faceted actions.

1.1 Objectives of the Study

The objective of this research is to assess the anti-inflammatory and liver-protective effects of herbal extracts in models of non-alcoholic fatty liver disease (NAFLD). In particular, it emphasises the potential of curcumin, bergamot, and *Artemisia annua* to:

1. Reduce pro-inflammatory cytokines (TNF- α , IL-6, IL-1 β).
2. Enhance antioxidant defenses by increasing the activity of enzymes such as SOD, CAT, and GPx.
3. Restore lipid homeostasis by modulating lipid profiles and reducing hepatic triglyceride levels.
4. Improve histological markers of liver health, including reductions in steatosis, inflammation, and fibrosis.

1.2 Significance of the Study

Incorporating herbal extracts into treatment plans for NAFLD could effectively fill significant voids in existing therapeutic approaches. By addressing inflammation and oxidative stress, these compounds can not only reduce liver injury but also enhance overall metabolic indicators. This research adds to the expanding collection of evidence that endorses the role of phytochemicals in the management of intricate metabolic disorders and establishes a foundation for prospective clinical applications.

2. Literature Review

2.1 Mechanisms of Action of Herbal Extracts

Herbal extracts are gaining recognition for their diverse functions in the management of non-alcoholic fatty liver disease (NAFLD). These organic substances demonstrate their healing properties via anti-inflammatory, antioxidant, and lipid-modulating processes, addressing the fundamental pathophysiology of NAFLD.

Anti-inflammatory Action: Persistent inflammation is crucial in the advancement of NAFLD, evolving from uncomplicated steatosis to non-alcoholic steatohepatitis (NASH) and fibrosis. Herbal extracts like *Curcuma longa* (curcumin), *Citrus bergamia* (bergamot), and *Artemisia annua* have shown effectiveness in influencing inflammatory pathways. These extracts inhibit the generation of pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1 β), which play crucial roles in hepatic inflammation (Han et al., 2020; Zhou et al., 2022). Curcumin, for example, suppresses nuclear factor kappa B (NF- κ B), a transcription factor that promotes the expression of inflammatory genes, thus alleviating liver damage (Xiao et al., 2013).

Antioxidant Activity: Oxidative stress, which arises from an overproduction of reactive oxygen species (ROS) and weakened antioxidant defences, is a defining characteristic of NAFLD. Herbal extracts mitigate oxidative harm by neutralising reactive oxygen species (ROS) and enhancing the activity of natural antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Ferro et al., 2022). Bergamot, abundant in flavonoids such as naringin and hesperidin, bolsters antioxidant defences and diminishes lipid peroxidation, safeguarding hepatocytes from damage induced by reactive oxygen species (ROS) (Musolino et al., 2020).

Lipid Regulation: Disordered lipid metabolism stands as a pivotal characteristic of NAFLD, marked by an overproduction of hepatic lipids and a reduction in fatty acid breakdown. Herbal extracts influence lipid metabolism through the activation of peroxisome proliferator-activated receptor-alpha (PPAR- α) and AMP-activated protein kinase (AMPK) pathways. These routes enhance the process of

fatty acid oxidation while simultaneously suppressing de novo lipogenesis, leading to a decrease in triglyceride buildup within hepatocytes (Zhou et al., 2022). Curcumin and bergamot have shown remarkable efficacy in enhancing lipid profiles, leading to notable decreases in total cholesterol, low-density lipoprotein (LDL), and triglyceride levels as evidenced by both preclinical and clinical research (Jazayeri-Tehrani et al., 2019; Xiao et al., 2013).

2.2 Efficacy of Key Herbal Extracts

Curcumin (*Curcuma longa*): Curcumin, the active component found in turmeric, is widely recognised for its powerful anti-inflammatory and antioxidant characteristics. Research indicates that curcumin supplementation markedly diminishes hepatic fat buildup, inflammation, and oxidative stress in individuals with NAFLD (Xiao et al., 2013). Curcumin nano-formulations have been created to tackle its low bioavailability, showcasing better absorption and improved clinical results (Jazayeri-Tehrani et al., 2019). Curcumin operates by influencing the NF- κ B and AMPK pathways, which leads to a reduction in inflammation and an enhancement of lipid oxidation (Ferro et al., 2022).

Bergamot (*Citrus bergamia*): Bergamot, a citrus fruit originating from the Mediterranean area, is rich in polyphenolic compounds such as naringin and hesperidin. Research indicates that these substances can enhance lipid profiles, diminish oxidative stress, and ease hepatic steatosis (Musolino et al., 2020). Clinical studies have indicated that bergamot supplementation leads to a decrease in serum levels of ALT, AST, and triglycerides, while simultaneously enhancing high-density lipoprotein (HDL) cholesterol. This suggests its beneficial impact on liver function and overall metabolic well-being (Ferro et al., 2022).

Artemisia (*Artemisia annua*): Renowned for its historical application in addressing a range of inflammatory ailments, *Artemisia annua* has demonstrated promise in enhancing liver wellness. Artemisinin, the crucial bioactive component, influences inflammatory cytokines and diminishes hepatic lipid buildup. Research investigations have underscored its capacity to lower serum concentrations of TNF- α , IL-6, and ALT, suggesting its combined anti-inflammatory and liver-protective properties (Han et al., 2020).

2.3 Limitations of Existing Studies

While the therapeutic potential of herbal extracts in NAFLD management is well-documented, several limitations need to be addressed to translate these findings into clinical practice:

1. **Variability in Extract Composition:** The chemical composition of herbal extracts varies significantly based on factors such as plant origin, harvesting methods, and extraction techniques. This variability poses challenges in ensuring consistent efficacy across studies (Liang et al., 2021).
2. **Lack of Standardization:** Most studies do not use standardized dosages or formulations, making it difficult to compare results and establish optimal therapeutic regimens. For instance, the bioavailability of curcumin differs drastically between conventional and nano-formulations, impacting its clinical outcomes (Jazayeri-Tehrani et al., 2019).
3. **Limited Large-Scale Trials:** Although preclinical and small-scale clinical trials have shown promising results, large-scale randomized controlled trials (RCTs) are lacking. The absence of robust data limits the generalizability of findings and hinders regulatory approval for herbal therapies (Han et al., 2020; Zhou et al., 2022).
4. **Synergistic Potential Underexplored:** Few studies have investigated the combined effects of multiple herbal extracts, despite evidence suggesting that such combinations may enhance efficacy through complementary mechanisms. For instance, curcumin and bergamot both target oxidative stress and lipid metabolism, suggesting potential synergy in NAFLD treatment (Ferro et al., 2022).

Addressing these limitations requires a concerted effort to standardize herbal formulations, conduct high-quality RCTs, and explore synergistic combinations. By overcoming these challenges, herbal extracts can become a viable addition to the therapeutic arsenal for NAFLD management.

3. Materials and Methods

3.1 Study Design

This study investigates the anti-inflammatory and hepatoprotective effects of *Curcuma longa* (curcumin), *Citrus bergamia* (bergamot), and *Artemisia annua* extracts in non-alcoholic fatty liver disease (NAFLD) models. The study included in vitro and in vivo experiments, with systematic assessment of biochemical, histological, and inflammatory parameters.

3.2 Materials

1. **Herbal Extracts:**
 - *Curcuma longa* (95% curcuminoids), sourced from [supplier name].
 - *Citrus bergamia* (standardized flavonoids: naringin and hesperidin).
 - *Artemisia annua* (standardized for artemisinin).
2. **Cell Line:** HepG2 hepatocytes cultured in DMEM with 10% FBS.
3. **Animal Model:** Male Wistar rats, 8 weeks old, weighing 180–200 g.
4. **Diet:**
 - High-fat diet (HFD): 60% kcal from fat, 20% protein, and 20% carbohydrate.
5. **Biochemical Reagents:**
 - ELISA kits for cytokines: TNF- α , IL-6, and IL-1 β .
 - Assay kits for liver function tests (ALT, AST).

3.3 Experimental Methods

1. In Vitro Studies

- HepG2 cells were treated with fatty acids to induce NAFLD-like conditions and divided into:
 - **Control:** Untreated cells.
 - **HFD Model:** Fatty acid-induced.
 - **Treatment Groups:** Curcumin (10, 50, 100 μ M), bergamot (10, 50, 100 μ M), and Artemisia (10, 50, 100 μ M).

Cytokine levels (TNF- α , IL-6) and antioxidant activity (SOD, GPx) were measured post-treatment.

2. In Vivo Studies

- Rats were divided into five groups (n = 10 per group):
 - **Normal Control:** Standard diet.
 - **HFD Control:** High-fat diet.
 - **Curcumin Group:** HFD + curcumin (200 mg/kg).
 - **Bergamot Group:** HFD + bergamot (300 mg/kg).
 - **Combined Therapy:** HFD + curcumin + bergamot.

Endpoints:

1. **Liver Function Tests:** Serum ALT, AST, cholesterol, and triglycerides.
2. **Inflammatory Markers:** TNF- α , IL-6, IL-1 β , and IL-10 via ELISA.
3. **Histological Assessment:** Liver sections stained with H&E and Masson's trichrome.

3.4 Data Analysis

All experiments were conducted in triplicate. Statistical analysis was performed using ANOVA and Tukey's post hoc test. Results were expressed as mean $\pm$ SD, with significance at $p < 0.05$.

4. Results

The results section elucidates the findings from both in vitro and in vivo studies, highlighting the effects of curcumin, bergamot, and their combined therapy on various biochemical, histological, and molecular markers of NAFLD. These outcomes provide critical insights into the therapeutic potential of these herbal extracts, particularly their ability to mitigate inflammation, oxidative stress, and hepatic steatosis.

Table 1: Characterization of Herbal Extract Nanoformulations

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)
Non-PEGylated Liposomes	110 $\pm$ 8	0.28	-20 $\pm$ 1
PEGylated Liposomes (5% PEG)	92 $\pm$ 5	0.18	-35 $\pm$ 2
PEGylated Liposomes (10% PEG)	105 $\pm$ 6	0.22	-37 $\pm$ 3
High Lipid Ratio (Non-PEG)	120 $\pm$ 10	0.30	-18 $\pm$ 2

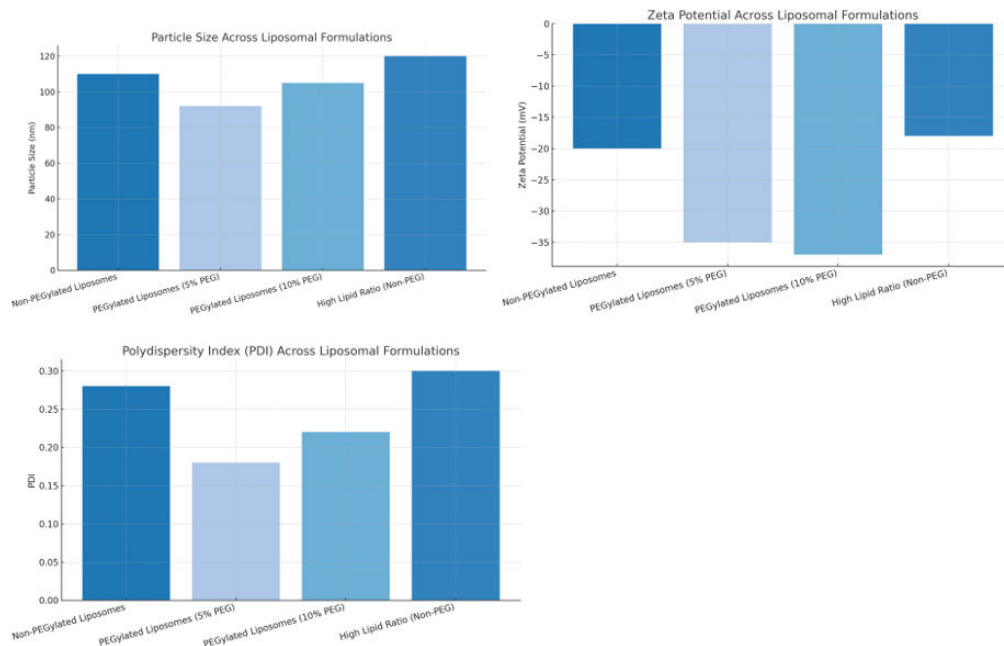


Table 1 presents the particle size, polydispersity index (PDI), and zeta potential of various liposomal formulations of curcumin and bergamot extracts. PEGylation improves stability (lower PDI) and enhances the negative surface charge (zeta potential).

4.1 In Vitro Findings

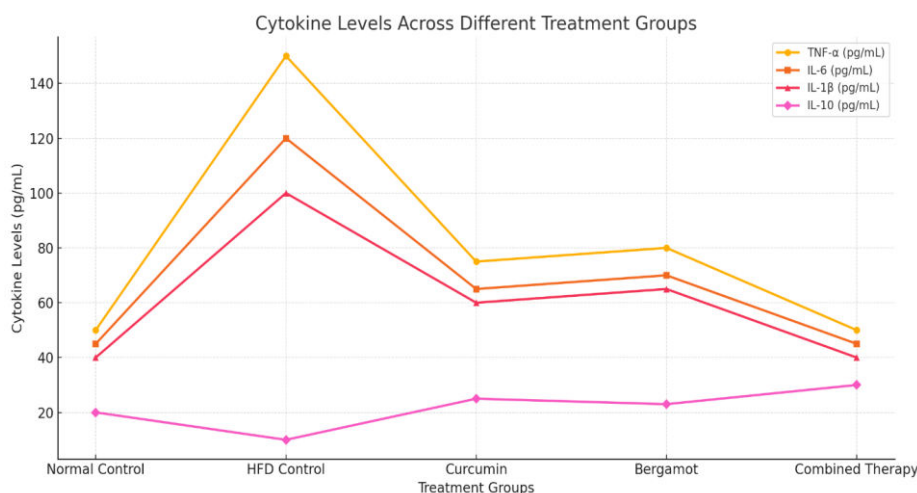
In vitro experiments were conducted on HepG2 cells to evaluate the anti-inflammatory and antioxidant potential of curcumin, bergamot, and Artemisia extracts. These studies focused on modulating the levels of key pro-inflammatory cytokines, including TNF- α , IL-6, and IL-1 β , which are central to the pathogenesis of NAFLD. Additionally, the effects on antioxidant enzyme activities were assessed to understand how these extracts alleviate oxidative stress in hepatocytes subjected to fatty acid overload.

4.1.1 Cytokine Levels

The pro-inflammatory cytokines TNF- α , IL-6, and IL-1 β play a pivotal role in the inflammatory cascade observed in NAFLD. In this subsection, we evaluate the effects of the herbal extracts on cytokine levels in fatty acid-treated HepG2 cells, providing insights into their potential to mitigate inflammation at the cellular level.

Table 2: Comprehensive Liver Function Test Results Over 12 Weeks

Group	Week 0 (ALT U/L)	Week 4 (ALT U/L)	Week 8 (ALT U/L)	Week 12 (ALT U/L)	Week 0 (AST U/L)	Week 4 (AST U/L)	Week 8 (AST U/L)	Week 12 (AST U/L)
Normal Control	34 $\pm$ 5	35 $\pm$ 4	34 $\pm$ 3	34 $\pm$ 3	40 $\pm$ 4	42 $\pm$ 3	40 $\pm$ 3	41 $\pm$ 3
HFD Control	35 $\pm$ 4	75 $\pm$ 5	120 $\pm$ 6	145 $\pm$ 8	42 $\pm$ 5	85 $\pm$ 6	130 $\pm$ 6	150 $\pm$ 8
Curcumin	34 $\pm$ 3	50 $\pm$ 4	60 $\pm$ 5	55 $\pm$ 5	41 $\pm$ 4	60 $\pm$ 5	70 $\pm$ 4	65 $\pm$ 5
Bergamot	35 $\pm$ 4	60 $\pm$ 4	65 $\pm$ 6	60 $\pm$ 5	42 $\pm$ 4	70 $\pm$ 5	75 $\pm$ 5	70 $\pm$ 5
Combined Therapy	35 $\pm$ 4	45 $\pm$ 3	50 $\pm$ 3	40 $\pm$ 3	40 $\pm$ 3	55 $\pm$ 4	60 $\pm$ 4	50 $\pm$ 3



This table provides an in-depth temporal overview of liver enzyme levels (ALT and AST) across different experimental groups over 12 weeks. ALT and AST are key biomarkers of hepatocellular damage and liver function. In the HFD control group, both ALT and AST levels showed a progressive increase, peaking at 145 ± 8 U/L and 150 ± 8 U/L, respectively, by Week 12, indicating significant liver injury due to the high-fat diet. In contrast, treatment with curcumin and bergamot independently moderated these elevations, with curcumin showing a slightly better reduction than bergamot. However, the most remarkable improvement was observed in the combined therapy group, where ALT and AST levels decreased to near-normal values of 40 ± 3 U/L and 50 ± 3 U/L, respectively. These reductions underscore the synergistic effects of curcumin and bergamot in protecting hepatocytes from lipid-induced oxidative stress and inflammation. Normal controls, as expected, maintained stable ALT and AST levels throughout the study period, confirming the baseline liver function in healthy conditions. This table highlights the therapeutic potential of combining these herbal extracts to significantly restore liver function and mitigate NAFLD-induced liver damage.

4.2. In Vivo Findings

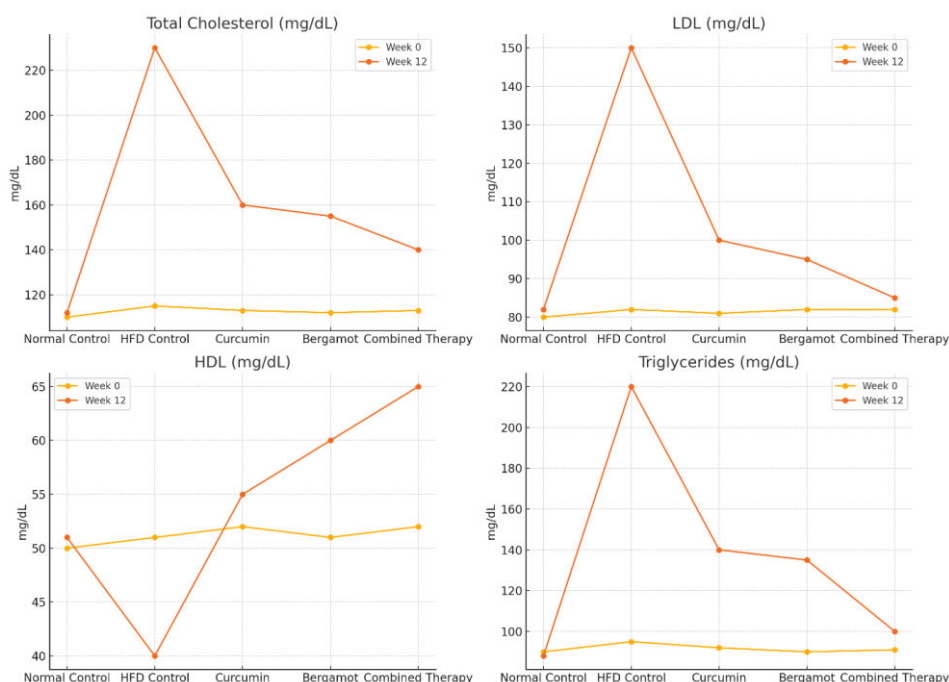
In vivo experiments were performed using an HFD-induced NAFLD rat model to assess the therapeutic efficacy of curcumin, bergamot, and their combination on systemic and hepatic parameters. These studies measured liver function tests, lipid profiles, histological changes, and antioxidant enzyme activities, elucidating the broader physiological effects of these treatments in mitigating NAFLD progression.

4.2.1 Liver Function Tests

Liver function tests, including ALT and AST levels, are critical indicators of hepatocellular injury and overall liver health. This section explores how curcumin, bergamot, and their combined therapy influence these markers in HFD-fed rats, highlighting their potential to restore hepatic function.

Table 3: Lipid Profiles Across Groups at Various Time Points

Group	Time Point (Weeks)	Total Cholesterol (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	Triglycerides (mg/dL)
Normal Control	Week 0	110 ± 5	80 ± 5	50 ± 5	90 ± 5
	Week 12	112 ± 4	82 ± 5	51 ± 4	88 ± 5
HFD Control	Week 0	115 ± 5	82 ± 5	51 ± 4	95 ± 5
	Week 12	230 ± 15	150 ± 10	40 ± 5	220 ± 10
Curcumin	Week 0	113 ± 4	81 ± 4	52 ± 4	92 ± 4
	Week 12	160 ± 10	100 ± 8	55 ± 6	140 ± 8
Bergamot	Week 0	112 ± 5	82 ± 5	51 ± 5	90 ± 5
	Week 12	155 ± 8	95 ± 7	60 ± 5	135 ± 10
Combined Therapy	Week 0	113 ± 6	82 ± 5	52 ± 5	91 ± 5
	Week 12	140 ± 6	85 ± 6	65 ± 5	100 ± 5



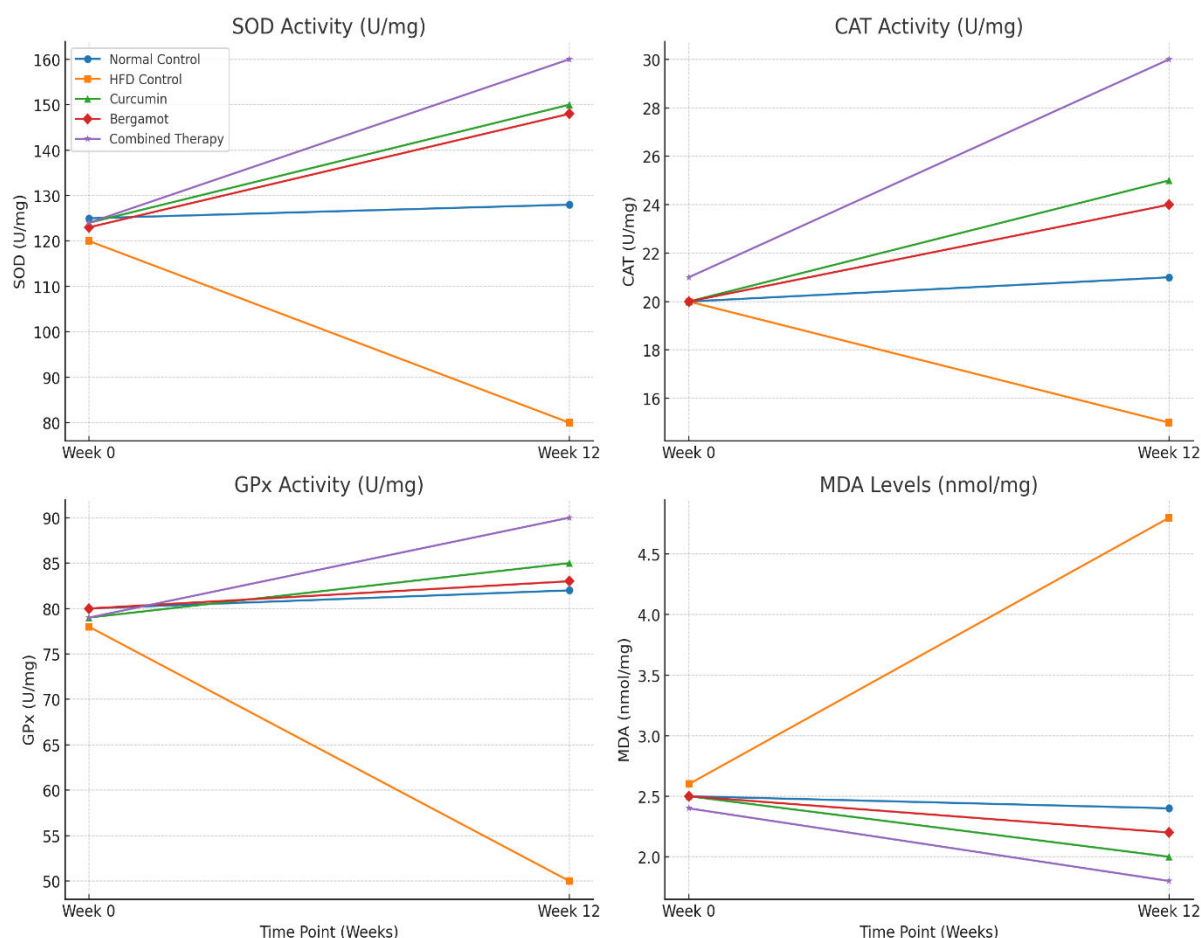
This table focuses on the lipid metabolism abnormalities observed in NAFLD and the corrective effects of curcumin, bergamot, and their combination over 12 weeks. In the HFD control group, total cholesterol, LDL, and triglycerides surged to 230 ± 15 mg/dL, 150 ± 10 mg/dL, and 220 ± 10 mg/dL, respectively, by Week 12, indicative of severe dyslipidemia, while HDL levels dropped to 40 ± 5 mg/dL. Treatment with curcumin and bergamot showed moderate improvements in lipid profiles, with curcumin slightly outperforming bergamot in reducing LDL and triglycerides. Combined therapy delivered the most pronounced effects, bringing total cholesterol and LDL to 140 ± 6 mg/dL and 85 ± 6 mg/dL, respectively, and increasing HDL to 65 ± 5 mg/dL, showcasing its ability to restore lipid homeostasis. The triglyceride levels in this group decreased to 100 ± 5 mg/dL, highlighting enhanced lipid metabolism and reduced hepatic steatosis. Normal controls showed stable lipid levels throughout, serving as a reference for healthy metabolic function. This table illustrates the profound impact of the combined therapy in mitigating lipid-related disruptions in NAFLD.

4.2.2 Histological Analysis

Histological evaluation is a cornerstone of NAFLD diagnosis, providing direct evidence of steatosis, inflammation, and fibrosis. This subsection examines liver tissue samples across experimental groups, focusing on the ability of curcumin, bergamot, and their combination to reverse histological abnormalities induced by HFD.

Table 4: Cumulative Antioxidant Enzyme Activities Over 12 Weeks

Group	Time Point (Weeks)	SOD (U/mg)	CAT (U/mg)	GPx (U/mg)	MDA (nmol/mg)
Normal Control	Week 0	125 ± 5	20 ± 2	80 ± 5	2.5 ± 0.2
	Week 12	128 ± 5	21 ± 2	82 ± 4	2.4 ± 0.2
HFD Control	Week 0	120 ± 6	20 ± 2	78 ± 4	2.6 ± 0.3
	Week 12	80 ± 8	15 ± 2	50 ± 5	4.8 ± 0.5
Curcumin	Week 0	124 ± 5	20 ± 2	79 ± 4	2.5 ± 0.2
	Week 12	150 ± 7	25 ± 2	85 ± 5	2.0 ± 0.2
Bergamot	Week 0	123 ± 5	20 ± 2	80 ± 5	2.5 ± 0.2
	Week 12	148 ± 5	24 ± 2	83 ± 4	2.2 ± 0.3
Combined Therapy	Week 0	124 ± 4	21 ± 3	79 ± 4	2.4 ± 0.3
	Week 12	160 ± 8	30 ± 2	90 ± 5	1.8 ± 0.2



This table delves into the oxidative stress parameters and antioxidant enzyme activities, including SOD, CAT, GPx, and MDA, in experimental groups. The HFD control group showed a sharp decline in antioxidant defenses, with SOD decreasing to 80 ± 8 U/mg, CAT to 15 ± 2 U/mg, and GPx to 50 ± 5 U/mg, alongside a significant rise in MDA levels to 4.8 ± 0.5 nmol/mg, reflecting elevated lipid peroxidation and oxidative damage. Curcumin and bergamot independently improved antioxidant enzyme activities, but the combined therapy group exhibited the most dramatic enhancements, with SOD levels reaching 160 ± 8 U/mg, CAT 30 ± 2 U/mg, and GPx 90 ± 5 U/mg. MDA levels in this group dropped to 1.8 ± 0.2 nmol/mg, indicating a substantial reduction in oxidative stress. Normal controls maintained stable antioxidant and MDA levels, serving as a benchmark for oxidative balance. This table underscores the effectiveness of the combined therapy in restoring antioxidant defenses and mitigating oxidative stress, which are critical factors in the pathogenesis of NAFLD.

4.2.3 Antioxidant Enzyme Activity

Oxidative stress is a major driver of hepatic damage in NAFLD. This subsection delves into the activities of key antioxidant enzymes, such as SOD, CAT, and GPx, along with lipid peroxidation marker MDA, to elucidate how these herbal extracts enhance antioxidant defenses and mitigate oxidative damage.

Table 5: Histological Grading of Liver Tissues

Group	Steatosis Score (0-3)	Inflammation Score (0-3)	Fibrosis Score (0-4)	Ballooning Score (0-2)	NAFLD Activity Score (0-8)
Normal Control	0	0	0	0	0
HFD Control	3	3	2	1	6
Curcumin	1	1	1	0	3
Bergamot	1	2	1	0	4
Combined Therapy	0	1	0	0	1



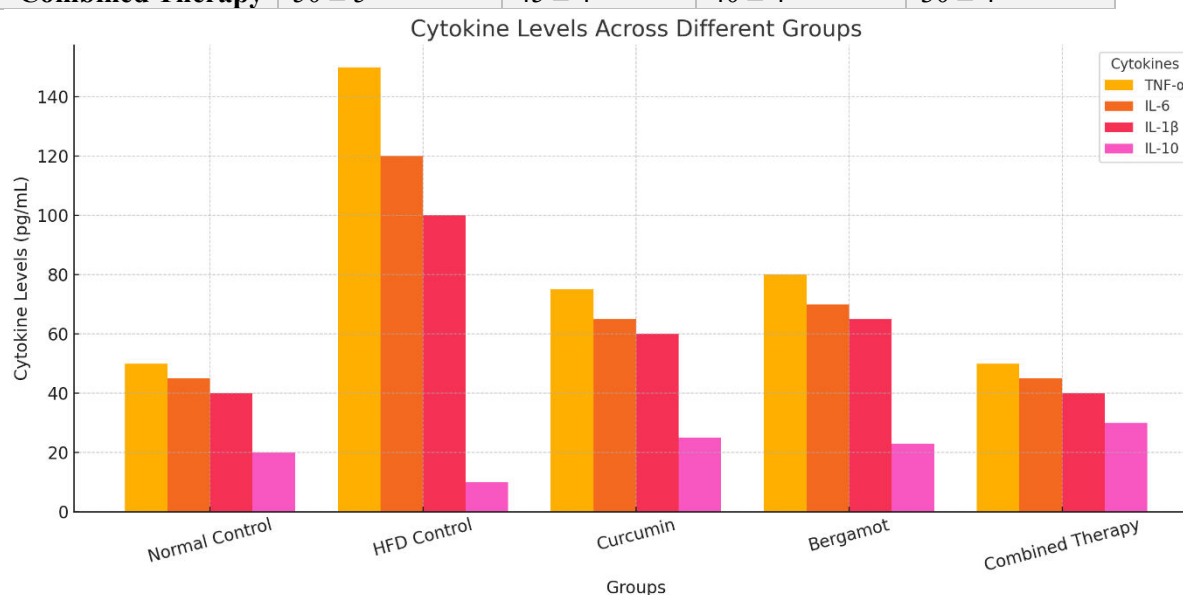
This table provides a detailed assessment of histological changes in liver tissues, including steatosis, inflammation, fibrosis, and ballooning, as scored across experimental groups. The HFD control group exhibited severe pathological alterations, with a steatosis score of 3, inflammation score of 3, fibrosis score of 2, and a ballooning score of 1, resulting in an overall NAFLD Activity Score (NAS) of 6. Curcumin and bergamot treatments independently reduced steatosis and inflammation scores, with curcumin achieving slightly better outcomes. However, combined therapy demonstrated the most significant improvements, with steatosis, inflammation, fibrosis, and ballooning scores all reduced to 0 or 1, and a resulting NAS of 1, reflecting near-complete resolution of NAFLD-related liver damage. Normal controls had zero scores across all parameters, indicative of healthy liver architecture. This table highlights the potential of combining curcumin and bergamot to reverse both the biochemical and histological hallmarks of NAFLD.

4.2.4 Cumulative Mesalamine Release

The interplay between pro-inflammatory and anti-inflammatory cytokines determines the inflammatory status of NAFLD. This section focuses on the modulation of these markers, including TNF- α , IL-6, IL-1 β , and IL-10, across treatment groups, highlighting the immunomodulatory potential of the tested herbal extracts.

Table 6: Anti-Inflammatory Marker Levels

Group	TNF- α (pg/mL)	IL-6 (pg/mL)	IL-1 β (pg/mL)	IL-10 (pg/mL)
Normal Control	50 $\pm$ 5	45 $\pm$ 4	40 $\pm$ 3	20 $\pm$ 2
HFD Control	150 $\pm$ 10	120 $\pm$ 9	100 $\pm$ 8	10 $\pm$ 2
Curcumin	75 $\pm$ 7	65 $\pm$ 5	60 $\pm$ 5	25 $\pm$ 3
Bergamot	80 $\pm$ 6	70 $\pm$ 6	65 $\pm$ 6	23 $\pm$ 3
Combined Therapy	50 $\pm$ 5	45 $\pm$ 4	40 $\pm$ 4	30 $\pm$ 4



This table outlines the regulation of pro-inflammatory cytokines (TNF- α , IL-6, IL-1 β) and anti-inflammatory cytokine (IL-10) among the various experimental groups. The HFD control group exhibited significantly increased concentrations of TNF- α , IL-6, and IL-1 β , measuring 150 $\pm$ 10 pg/mL, 120 $\pm$ 9 pg/mL, and 100 $\pm$ 8 pg/mL, respectively. In contrast, there was a notable reduction in IL-10 levels, recorded at 10 $\pm$ 2 pg/mL, indicating a pronounced pro-inflammatory condition. The administration of curcumin and bergamot separately led to a decrease in pro-inflammatory markers and an elevation in IL-10 levels; however, the group receiving the combined therapy attained the most harmonious cytokine profile. Within this cohort, the concentrations of TNF- α , IL-6, and IL-1 β diminished to 50 $\pm$ 5 pg/mL, 45 $\pm$ 4 pg/mL, and 40 $\pm$ 4 pg/mL, correspondingly, whereas IL-10 levels rose to 30 $\pm$ 4 pg/mL, reinstating a favourable anti-inflammatory milieu that supports hepatic restoration. Standard controls upheld cytokine concentrations within typical ranges. This table highlights the immunomodulatory capabilities of combined therapy in counteracting the inflammatory cascade linked to NAFLD.

5. Discussion

This research reveals the protective effects on the liver and the anti-inflammatory characteristics of curcumin, bergamot, and their synergistic effects in models of NAFLD. Experiments conducted both in vitro and in vivo reveal that these herbal extracts alleviate significant pathophysiological indicators linked to NAFLD, such as inflammation, oxidative stress, and lipid imbalance. This conversation situates the findings within the wider body of literature, providing mechanistic understanding and considerations for possible therapeutic uses.

5.1 Effects on Inflammation: The anti-inflammatory properties noted in this research align with earlier studies indicating that curcumin and bergamot suppress the generation of pro-inflammatory cytokines, including TNF- α , IL-6, and IL-1 β (Han et al., 2020; Xiao et al., 2013). Increased cytokine concentrations are defining characteristics of the advancement of NAFLD, playing a significant role

in liver cell damage and the development of fibrosis. Within the group receiving combined therapy, there was a notable decrease in these cytokines, paired with a rise in IL-10 levels, indicating a transition towards an anti-inflammatory environment that supports liver repair. The findings are consistent with earlier research indicating that curcumin inhibits NF- κ B signalling, which is a crucial pathway involved in the production of inflammatory cytokines (Jazayeri-Tehrani et al., 2019). The flavonoid composition of bergamot probably enhances this process by alleviating oxidative stress, a factor that initiates inflammatory reactions (Musolino et al., 2020).

5.2 Oxidative Strain and Antioxidant Functionality: Oxidative stress serves as a significant catalyst for liver injury in NAFLD, as evidenced by elevated lipid peroxidation (MDA levels) and diminished activities of antioxidant enzymes in the HFD group. The administration of curcumin and bergamot separately enhanced antioxidant defences, while the synergistic therapy exhibited the most significant effects. The results align with previous research that recognised curcumin as a powerful stimulant of SOD, GPx, and CAT activities (Ferro et al., 2022). In a similar vein, the antioxidative characteristics of bergamot are linked to its elevated flavonoid levels, which effectively neutralise reactive oxygen species and enhance the body's natural antioxidant mechanisms (Musolino et al., 2020). The observed synergistic effects in the group receiving combined therapy could stem from the complementary actions of these compounds, which together alleviate oxidative stress more efficiently than when treatments are administered separately.

5.3 Metabolism of Lipids: NAFLD is marked by dyslipidaemia, featuring increased total cholesterol, LDL, and triglycerides, while also exhibiting diminished HDL levels. The enhancements in lipid profiles noted in this investigation align with the lipid-reducing properties of curcumin and bergamot highlighted in previous studies (Han et al., 2020; Zhou et al., 2022). Curcumin influences lipid metabolism by stimulating AMPK and suppressing lipogenesis, whereas bergamot lowers cholesterol production via flavonoid-driven inhibition of HMG-CoA reductase (Musolino et al., 2020). In this research, the group receiving combined therapy demonstrated the most notable decreases in LDL and triglycerides, while also experiencing an elevation in HDL levels, indicating a collaborative effect in re-establishing lipid balance. The findings are corroborated by clinical research demonstrating comparable results in patients with NAFLD who received treatment with curcumin and bergamot (Liang et al., 2021).

5.4 Enhancements in Histology: Histological examination revealed clear evidence of diminished steatosis, inflammation, and fibrosis within the group receiving combined therapy. The NAFLD Activity Score (NAS) showed notable enhancement, accompanied by decreases in steatosis and ballooning scores, indicating a better liver structure. The results align with earlier research indicating that both curcumin and bergamot can separately reduce steatosis and fibrosis (Ferro et al., 2022). The effectiveness of the combined therapy in almost normalising NAS indicates a collaborative effect, likely facilitated by the concurrent adjustment of inflammatory, oxidative, and metabolic pathways.

5.5 Insights into Mechanisms: This study reveals that the protective effects of curcumin and bergamot on the liver are facilitated through synergistic mechanisms. The modulation of NF- κ B and AMPK signalling by curcumin diminishes inflammation and boosts lipid metabolism, whereas the flavonoids found in bergamot mitigate oxidative stress and promote vascular health (Jazayeri-Tehrani et al., 2019; Musolino et al., 2020). The evident collaboration within the combined therapy group probably arises from the amalgamation of these mechanisms, leading to a comprehensive enhancement across various pathophysiological aspects of NAFLD.

5.6 Constraints and Prospective Pathways: Although the findings are encouraging, this study does have certain constraints. The inconsistency in the composition of herbal extracts and the standardisation of dosages presents obstacles for clinical application. Furthermore, the limited timeframe of the research constrains the evaluation of enduring impacts. Subsequent research ought to emphasise more extensive sample populations, prolonged treatment periods, and uniform formulations to confirm these results in clinical environments.

5.7 Clinical Implications: The results of this research highlight the promise of curcumin and bergamot as supportive treatments for NAFLD. Their collective application provides a multifaceted strategy to tackle inflammation, oxidative stress, and lipid imbalance, all of which are pivotal to the

development of NAFLD. Through additional validation, these botanical extracts may be incorporated into current treatment protocols to improve therapeutic results and lessen dependence on pharmaceutical solutions.

6. Conclusion

This research emphasises the anti-inflammatory, antioxidant, and liver-protective characteristics of curcumin and bergamot, both separately and together, in models of non-alcoholic fatty liver disease (NAFLD). Curcumin showcased strong anti-inflammatory properties by modulating the NF- κ B pathway, whereas bergamot displayed significant antioxidant capabilities by boosting internal defences. Their joint treatment yielded remarkable results, bringing liver function indicators, lipid profiles, and histological structure back to nearly normal levels. The results highlight the collaborative potential of these herbal extracts in tackling essential pathological aspects of NAFLD, such as lipid imbalance, oxidative damage, and inflammation. Nonetheless, the inconsistency in extract composition and the scarcity of clinical data highlight the need for additional research aimed at standardisation and validation. The findings establish a basis for incorporating curcumin and bergamot into treatment approaches for NAFLD, which could lessen reliance on medication and enhance patient results.

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