

RESEARCH ARTICLE

Effects of Tea Formulated From *Citrus Aurantifolia*, *Syzygium Aromaticum* and *Mentha Piperita L* on Weight and Biochemical Parameters of Male Obesed Rats

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Abstract

The rising prevalence of obesity, associated with metabolic disorders and organ dysfunction, necessitates the exploration of natural, plant-based interventions. This study investigated the effects of a polyherbal tea formulated from lime (*Citrus aurantifolia*), clove (*Syzygium aromaticum*), and peppermint (*Mentha piperita L.*) on obesity-related parameters in male Wistar rats. The specific objectives were: (i) to formulate and evaluate the nutritional and phytochemical components of the tea, (ii) to assess its anti-obesity effects on weight and lipid profiles, and (iii) to evaluate its influence on hepatic and renal function. Fresh limes, dried cloves, and peppermint leaves were processed following WHO guidelines, dehydrated, powdered, and blended in three ratios. Phytochemical screening revealed that L3 (25% lime, 25% mint, 50% clove) exhibited the highest concentrations of bioactive compounds (Phenols, Tannins, Saponins and Flavonoids) was selected for infusion. Quantitative analysis showed the tea contained low carbohydrates and fats, preserved vitamin C (~2.9 mg/g), very low moisture (<1.2%), and high phenols, flavonoids, tannins, then saponins. Thirty (30) male Wistar rats (150–180 g) were randomly assigned into six groups of 5 rats each: normal control, obese control, atorvastatin (10 mg/kg) reference, and three treatment groups receiving graded tea doses (75, 150, and 300 mg/kg). Obesity was induced by an 8-week high-fat diet, followed by 30 days of treatment. Results demonstrated that high-fat feeding significantly increased total cholesterol, triglycerides, LDL-C, coronary risk index, atherogenic index, creatinine, and liver enzymes (ALAT, ASAT), while reducing HDL-C. Treatment with lime–mint–clove tea improved these alterations in a dose-dependent manner. The 150 mg/kg dose produced the most consistent hypolipidemic, hypoweighting, hepatoprotective, and nephroprotective effects, comparable in several parameters to atorvastatin group of rats. In conclusion, the formulated lime–mint–clove tea demonstrated significant anti-obesity, lipid-lowering, and reducing ALAT, was associated with a significant increase in creatinine levels, suggesting potential renal toxicity and lack of side effects during treatment in obese male rats, highlighting its potential as a functional food and natural alternative in obesity management. These findings provide a scientific basis for the traditional use of lime, mint, and clove in managing metabolic disorders and support further research into their clinical applicability.

Keywords: *Citrus Aurantifolia*; *Syzygium Aromaticum*; *Mentha Piperita L* Tea; Weight; Biochemical Parameters; Obesed Rats.

Introduction

Obesity is a multifactorial metabolic disorder characterized by excessive fat accumulation that poses major health risks. It is commonly assessed using the Body Mass Index (BMI), with a BMI ≥ 30 classified as obese. Globally, obesity has reached epidemic levels—affecting about 13% of adults (over 650 million people) in 2022—and is projected to exceed 1.12 billion by 2035 if trends persist [1]. In Africa, obesity rates continue to rise, especially in urban populations, with prevalence rates reported at 17.8% in Ghana, 30% in South Africa, and 33.7% in Nigeria [2]. In Cameroon, adult obesity increased from 4.9% in 2000 to 9.5% in 2016, reaching up to 49% in urban women [2].

Male obesity arises primarily from an imbalance between caloric intake and energy expenditure. Increasing evidence indicates that dietary phytochemicals can influence metabolism and promote weight control. Polyphenols from tea are known to enhance fat oxidation and improve insulin sensitivity [3]. Similarly, limes (*Citrus aurantifolia*), cloves (*Syzygium aromaticum*), and mint (*Mentha piperita*) contain bioactive compounds that may aid obesity management through antioxidant, anti-inflammatory, and metabolic-regulating effects.

Limes, rich in vitamin C and flavonoids, help reduce oxidative stress and modulate lipid metabolism, contributing to body weight reduction [4]. Cloves, abundant in eugenol, have demonstrated antioxidant and anti-inflammatory effects that promote energy expenditure and improve gut microbiota [5]. Mint, particularly peppermint, exhibits appetite-suppressing and thermogenic properties through menthol, enhancing digestion and metabolism [6].

The combination of these botanicals in a tea infusion may yield synergistic effects, targeting multiple metabolic pathways linked to obesity. Previous studies have shown that plant polyphenols can act additively to improve lipid metabolism and metabolic health [7, 6]. Lime, mint, and clove each have known anti-obesity properties, but the synergistic effect of their combined formulation needs to be made clearer.

Hence, this study evaluates the synergistic effect of the combination of tea formulated from *Citrus aurantifolia*, *Syzygium aromaticum*, and *Mentha piperita* on obese male Wistar rats. It aims to assess the tea formulated potential to improve lipid profiles and liver function compared with a standard anti-obesity drug, providing insights into natural, plant-based approaches to managing obesity.

Materials and Methods

Collection and Preparation of Limes-Mint-Clove (LMC) Tea

Fresh limes (*Citrus aurantifolia*) and dried whole cloves (*Syzygium aromaticum*) were purchased from Bamenda Main Market, Northwest Region of Cameroon, while fresh peppermint leaves (*Mentha piperita* L.) were harvested early in the morning from Bafukum, Bambili.

The preparation of LMC tea followed standardized herbal processing procedures as outlined by the World Health Organization. Limes were thoroughly washed, juiced, and the juice stored under freezing conditions. The peels were dehydrated at a controlled temperature for 72 hours, then milled into fine powder. Mint leaves were similarly washed, sliced, dehydrated, and pulverized into powder to preserve volatile and phenolic compounds [9]. Dried cloves were directly milled into powder according to conventional herbal processing methods. Comparable low-temperature drying and milling approaches have been reported by [10, 11], ensuring product stability and retention of bioactive constituents. All powdered samples and lime juice were stored in airtight containers to prevent contamination and degradation.

Three formulations of the powdered mixture were prepared as follows:

L1: 50 g lime + 25 g mint + 25 g clove

L2: 25 g lime + 50 g mint + 25 g clove

L3: 25 g lime + 25 g mint + 50 g clove

Each formulation was homogenized thoroughly and subjected to phytochemical screening for flavonoids, phenols, tannins, and saponins. For extraction, 400 g of the homogeneous powder was placed in a clean vessel and macerated in distilled water at a 10:1 solvent-to-solid ratio (4 L of water for 400 g of powder), along with the previously extracted lime juice. The sealed mixture was left to macerate at room temperature (20–25 °C) for 72 hours, with intermittent manual stirring to improve extraction efficiency. The filtrate was separated using cheesecloth and Whatman No. 1 filter paper. The plant residue (marc) was re-macerated twice for 24 hours each, and all filtrates were combined to maximize extraction yield [12].

The pooled extract was first refrigerated to maintain freshness, then concentrated into a dry tea powder by oven drying at 50 °C for seven days [13]. The process yielded approximately 28 g of dry LMC tea powder, which was stored in airtight containers until further use.

Experimental Animals and Diet

Thirty (30) male Wistar albino rats weighing 150–180 g was obtained from the Animal House at the University of Bamenda. Only male rats were selected to avoid hormonal influences associated with the estrous cycle in females, which may affect weight and biochemical parameters. The animals were housed in separate cages and kept at room temperature at 12 hours cycle of light and darkness. They had free access to feed and water ad libitum. All animals were acclimatized to the working environment for 1 week before they were used for the experiment [14].

Obesity was induced by feeding the rats a high-fat diet for 8 weeks. Aside the normal control group (fed on a normal diet composed of 70% corn flour, 10% palm oil, 10% soya beans flour, 8% fish powder, 1% bone powder and 1% vitamin), the other rats (experimental groups) were given high fat diet (composed of 50% corn flour, 30% palm oil, 10% soya beans flour, 8% fish powder, 1% bone powder and 1% vitamin) daily for 56 days. The composition of the experimental diets are shown in table 2. The obese status of the animals was confirmed with lee's index ≥ 300 [14]. Obese rats were partitioned into 6 groups of 5 animals each, orally treated by gavage for 30 days with either distilled water (10ml/kg), 75, 150 or 300 mg/kg of tea, or 10mg/kg of Atorvastatin (the reference compound). The choice of doses was based on previous studies demonstrating the high rates of total polyphenols, tannins, flavonoids of safety and efficacy of polyphenol-rich plant extracts [15, 31, 14]. The group of normal diet control rats was left untreated, and body weights of all animals were recorded every 3 days.

The table below shows the nutrient composition of diets (g/kg) fed to the rats over an 8 weeks period to induce obesity.

Table 1: Nutrient Composition of Diets (g/kg)

Nutrient	Negative Control (Normal Diet)	Positive Control (HFD+ DW (water)	Atorvastatin Group (HFD +10 mg/kg Atvn)	Tea Group (HFD + 75 mg/kg tea)	Tea Group (HFD + 150 mg/kg Tea)	Tea Group (HFD + 300 mg/kg Tea)
Corn Flour(g)	700	500	500	500	500	500
Palm Oil(g)	100	300	300	300	300	300
Soya Bean Flour(g)	100	100	100	100	100	100

Fish Powder(g)	80	80	80	80	80	80
Bone Powder(g)	10	10	10	10	10	10
Vitamin Mix(g)	10	10	10	10	10	10
Atorvastatin mg/kg	-	-	10	-	-	-
Tea Extract mg/kg	-	-	-	75	150	300

Biochemical Analysis and Measurements

At the end of the treatment, the animals were fastened overnight, then anesthetized using diazepam (10mg/kg) and sacrificed. Capillary blood was collected and Serum was separated by centrifugation at 3000 rpm for 15 minutes and stored at -20 °C until use for subsequent biochemical analysis. The organ liver and kidney were dissected out and weighed. Homogenates (20% w/v, in phosphate buffer (pH 7.4, 50 mmol)) was prepared from the liver and used for the assessment of ALAT and ASAT [14].

Statistical Analysis

All data were expressed as mean \pm standard deviation. Statistical analysis was performed using SPSS version 25.0. One-way analysis of variance (ANOVA) was used to compare differences among groups, followed by Tukey's post hoc multiple comparison test. Significance was considered at $p < 0.05$ [15].

Results and Discussion

Effects of LMC Tea on Weight Gain

Treatment with 10 mg/kg Atorvastatin or 75 mg/kg, 150 mg/kg, 300 mg/kg LMC tea reduced the body weight of HFD obese rats, as compared with the obese untreated group (figure 1). Notably the 300 mg/kg had the most weight reducing effect.

As shown in table 2, all doses of the LMC tea and the reference compound Atorvastatin significantly ($p < 0.05$) reduced the Lee index as compared to the untreated HFD animals. But, none of the treatment totally normalize the Lee index with respect to the normal diet control group.

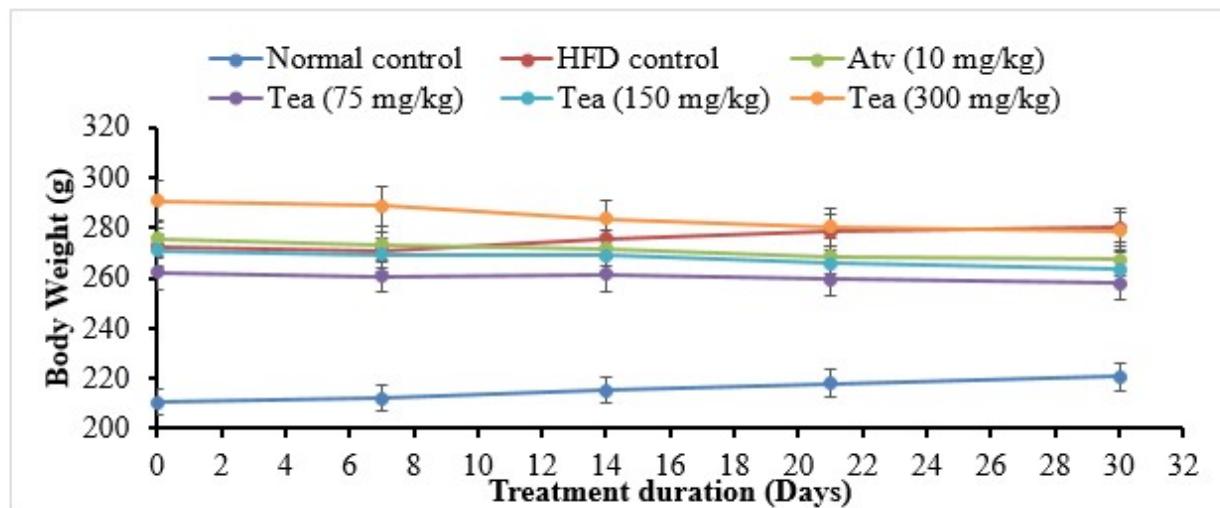


Figure 1: Variation in the Body Weights of Obese Rats With Different Treatments, Values Represent Mean \pm SD Of 5 Animals Per Group. Atvn: Atorvastatin, HFD: High-Fat Diet-Diet,

Lee index of animals during the experimentation

Table 2: Lee Index of Animals During Experimentation

Groups	Lee index at the beginning of treatment	Lee index at day 30
Normal control	288.0 ± 4.2 ^a	293.3 ± 5.1 ^a
HFD + DW (10 ml/kg)	345.2 ± 7.8 ^b	362.1 ± 7.5 ^d
HFD + Atorvastatin (10 mg/kg)	346.1 ± 6.9 ^b	331.4 ± 6.8 ^c
HFD + LN (75 mg/kg)	344.3 ± 7.1 ^b	335.2 ± 7.0 ^c
HFD + LN (150 mg/kg)	346.0 ± 6.3 ^b	322.7 ± 6.1 ^b
HFD + LN (300 mg/kg)	345.5 ± 5.4 ^b	315.2 ± 5.3 ^b

Values are presented as mean ± SD of 5 animals per group. Values not sharing a common letter differ significantly with the normal control and with each other (p < 0.05, Tukey's pair-wise comparison). DW: distilled water, HFD: high-fat-diet, LN: tea formulated

Effects of tea on lipidermic parameters and cardiovascular indices in HFD-induced obese rats

Lipidermic parameters of the rats after treatment with LMC tea or Atorvastatin is shown in table 3. The significantly increased serum TC, TG, LDL-C and VLDL-C concentrations as well as AI and CRI, while it reduced (p < 0.05) serum HDL-C levels. Treatment of rats with 150 mg/kg and 300 mg/kg LMC tea normalized these parameters as compared with the normal control animals.

Table 3: Effects of Tea (LN) on Lipid Profile and Atherogenic Indices in Wistar Rats

Lipid Profile	Normal Control	HFD Control	Atorvastatin (10 mg/kg)	Tea Dose 1 (75 mg/kg)	Tea Dose 2 (150 mg/kg)	Tea Dose 3 (300 mg/kg)
TC (mg/dL)	70.04 ± 5.17 ^a	136.12 ± 7.69 ^c	42.53 ± 11.09 ^b	114.37 ± 28.31 ^c	48.91 ± 1.81 ^b	64.11 ± 14.73 ^{ab}
TG (mg/dL)	51.39 ± 7.12 ^a	105.29 ± 4.79 ^c	33.72 ± 8.09 ^a	44.55 ± 9.01 ^a	27.62 ± 7.13 ^a	40.54 ± 14.03 ^a
LDL-C (mg/dL)	40.48 ± 2.63 ^a	118.36 ± 6.33 ^c	24.75 ± 4.79 ^a	82.75 ± 4.78 ^c	21.49 ± 6.72 ^a	35.06 ± 7.45 ^{ab}
HDL-C (mg/dL)	50.80 ± 4.20 ^a	21.31 ± 0.96 ^c	50.28 ± 1.38 ^a	18.68 ± 3.05 ^c	20.21 ± 3.89 ^{ab}	28.35 ± 3.80 ^b
VLDL-C (mg/dL)	21.31 ± 0.96 ^c	10.28 ± 1.42 ^a	16.28 ± 2.88 ^b	6.74 ± 1.61 ^a	2.21 ± 0.86 ^a	8.11 ± 2.81 ^b
CRI	1.38 ± 0.04 ^a	7.48 ± 1.52 ^c	1.28 ± 0.43 ^a	4.55 ± 1.40 ^b	3.27 ± 0.64 ^b	2.34 ± 0.74 ^{ab}
AI	0.80 ± 0.09 ^a	10.28 ± 1.42 ^c	1.01 ± 0.63 ^a	4.55 ± 1.40 ^b	3.27 ± 0.64 ^b	1.27 ± 0.39 ^{ab}

Values are expressed as mean ± standard deviation (n = 5). Values not sharing a common letter differ significantly (p < 0.05) when compared to the normal control group according to one-way ANOVA followed by Tukey's post hoc test. Identical superscripts indicate no significant difference. TC = total cholesterol, TG = triglycerides, LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol, VLDL-C = very-low-density lipoprotein cholesterol, CRI = coronary risk index, AI = atherogenic index.

Effects of Tea on Rat Creatinine

The following Table 4 shows the creatinine level. This rate provides information on the kidney health of rats that received tea.

Table 4: Creatinine Profile of HFD-Induced Obese Wistar Rats Upon Different Treatments

Parameter	Normal control (G1)	Obese control (G2)	Atorvastatin 10 mg/kg (G3)	Tea dose 1 (75 mg/kg, G4)	Tea dose 2 (150 mg/kg, G5)	Tea dose 3 (300 mg/kg, G6)
Creatinine (mg/L)	26.33 ± 3.13 ^a	68.57 ± 8.01 ^c	26.78 ± 6.34 ^a	42.86 ± 8.02 ^b	30.00 ± 5.25 ^{ab}	28.29 ± 3.83 ^a

Values represent mean±SD of 5 animals per group. Values not sharing a common letter differ significantly (p<0.05).

Effects of Tea on Markers of Harmfulness (Alat, Asat) in Rat Liver

The following Table 5 shows transaminase levels (ALAT, ASAT). These rates provide information on the liver health status of rats that received tea (limes, mint, cloves).

Table 5: Effects of Tea Administration on Liver Function Markers (ALAT and ASAT) in Obese Male Wistar Rats

Parameters (U/L)	Normal Control	HFD Control	Atorvastatin (10 mg/kg)	Tea dose 1 (75 mg/kg)	Tea dose 2 (150 mg/kg)	Tea dose 3 (300 mg/kg)
ALAT (GPT)	22.81±5.46 ^{ab}	29.40±5.72 ^b	14.44±10.16 ^a	44.91±8.78 ^c	22.05±5.88 ^{ab}	29.75±4.46 ^b
ASAT (GOT)	15.70±2.34 ^b	31.15±5.99 ^c	19.25±6.43 ^b	12.83±1.90 ^a	11.38±2.63 ^a	14.70±1.99 ^{ab}

Values are expressed as mean ± SD (n = 5). Means in the same row with different superscript letters (a, b, c) differ significantly (p<0.05). ALAT: Alanine aminotransferase; ASAT: Aspartate aminotransferase; HFD: High-fat diet.

Discussion

The nutritional and phytochemical analyses of the formulated tea revealed distinctive variations influenced by the proportions of lime, mint, and clove. Carbohydrate levels were generally low, with a slight decline in the clove-rich formulation (L3), reflecting the low sugar contribution of mint and clove compared to lime. Moisture content (1.1–1.2%) was within recommended limits (<6%), confirming adequate drying and product stability. Vitamin C was retained at approximately 2.9 mg/g, attributed to the citrus component and the partial resilience of ascorbic acid during drying. Crude fat content was minimal (~0.3%), consistent with reports on low-lipid herbal teas.

Phytochemical profiling indicated high total phenolic content across all formulations, slightly elevated in clove-enriched samples due to eugenol, a strong antioxidant. Flavonoid levels remained relatively stable, suggesting preservation of bioactivity despite variations in ingredient ratios. Tannin levels were notably high (~60%), exceeding those in conventional teas, likely reflecting clove-derived polyphenols that enhance antioxidant and anti-obesity effects but may influence nutrient absorption. Saponins were present at appreciable levels, further contributing to lipid-lowering, antioxidant, and anti-inflammatory properties. Collectively, the formulations demonstrated a unique nutritional and phytochemical profile characterized by low macronutrients, preserved vitamin C, and rich bioactive content, indicating synergistic potential against obesity.

In the present study, HFD-fed rats developed marked hypercholesterolemia (136.12 mg/dL) relative to the normal control (70.04 mg/dL), whereas atorvastatin and the formulated tea—particularly Dose 2 (48.91 mg/dL) and Dose 3 (64.11 mg/dL) sig-

nificantly reduced TC levels. Similar reductions in total cholesterol have been reported in studies where polyphenol-rich *Mentha* extracts and peppermint preparations improved lipid profiles in rodent models of diet-induced dyslipidemia [16]. Clove extracts, rich in eugenol, flavonoids and tannins, have also demonstrated significant TC-lowering effects through modulation of hepatic cholesterol synthesis and enhancement of antioxidant capacity [17, 18]. The strong hypocholesterolemic effect observed with the tea can be attributed to the bioactive compounds of lime, mint and cloves. Citrus peel flavonoids and essential oils inhibit hepatic HMG-CoA reductase and up-regulate LDL-receptor activity, thereby reducing circulating cholesterol [19]. Additionally, tannins present in all three ingredients can bind dietary lipids in the intestine and inhibit pancreatic lipase activity, thus limiting cholesterol absorption. These mechanisms collectively support the significant TC reductions observed in the tea-treated groups, consistent with published findings across citrus, mint and clove studies [6].

Triglyceride levels increased markedly in HFD-fed rats (105.29 mg/dL) relative to normal controls (51.39 mg/dL), consistent with diet-induced hyperlipidemia. Treatment with the formulated tea significantly lowered TG levels, with Dose 2 showing the greatest reduction (27.62 mg/dL), comparable to patterns reported for *Mentha* and peppermint extracts, which reduced TG in rodent models by modulating hepatic lipid metabolism [16, 21]

The TG-lowering effect can be explained by the polyphenols and tannins in lime, mint and cloves, which inhibit pancreatic lipase, thereby reducing intestinal fat absorption—a mechanism widely described for polyphenol-rich plant extracts [18]. Citrus peel flavonoids further contribute by down-regulating lipogenic enzymes and enhancing fatty acid oxidation, leading to decreased VLDL-TG secretion. The magnitude of TG reduction observed in the tea-treated groups is consistent with previous findings from citrus peel, clove and mint extract studies, demonstrating the capacity of these botanicals to reverse HFD-induced hypertriglyceridemia [6].

LDL-C rose sharply in HFD-fed rats (118.36 mg/dL), indicating typical diet-induced dyslipidemia, whereas atorvastatin and the formulated tea significantly countered this rise, with Dose 2 producing the lowest LDL-C (21.49 mg/dL). Comparable LDL-lowering effects have been reported in studies using *Mentha* species and peppermint extract, where LDL reduction was attributed to polyphenol-mediated modulation of hepatic cholesterol metabolism [21]. Lime peel flavonoids and essential oils also inhibit HMG-CoA reductase and enhance LDL receptor expression, increasing hepatic uptake of circulating LDL particles. Clove extracts exhibit similar actions, as their rich phenolic and tannin content reduces LDL synthesis and improves antioxidant defenses, limiting LDL oxidation. These combined mechanisms explain the potent LDL-lowering effect of the tea, aligning closely with outcomes reported for citrus, mint and clove-based interventions [22].

HDL-C decreased substantially in HFD-fed rats (21.31 mg/dL) compared to normal controls (50.80 mg/dL), reflecting impaired reverse cholesterol transport commonly seen in obesity models. Although atorvastatin restored HDL toward normal, the tea showed a dose-dependent yet modest improvement, with the highest dose (28.35 mg/dL) producing partial recovery. Similar variability has been reported in studies using *Mentha* extracts and citrus peel, where HDL increases were modest and dependent on dose and treatment duration metabolism [21]. The presence of flavonoids in lime, mint and cloves may enhance HDL stability and function by improving antioxidant activity and preserving apoA-I and paraoxonase activity, but elevation in HDL concentration is generally slower and less pronounced than reductions in LDL and TG (3–7). Therefore, the partial HDL restoration observed agrees with previous reports showing that botanical extracts improve HDL quality more readily than its circulating concentration [22].

VLDL-C (derived from TG) increased significantly in HFD-fed rats and declined following tea administration, with Dose 2 producing the lowest VLDL-C, consistent with its strong TG-lowering effect. The reduction in VLDL is expected because hepatic VLDL production depends on TG availability; thus, tannin-mediated inhibition of lipid absorption and polyphenol-driven suppression of hepatic lipogenesis directly limit VLDL secretion. Similar VLDL reductions have been reported in mint extract and

citrus peel studies, where lowered TG levels corresponded with reduced circulating VLDL particles. These findings support the conclusion that the combined phytochemicals of lime, mint and cloves effectively improve hepatic lipid handling and reduce VLDL output [23].

CRI increased markedly in HFD-fed rats (7.48) due to elevated LDL and reduced HDL; however, the formulated tea significantly improved this atherogenic index, especially at Dose 3 (2.34), reflecting a shift toward a less atherogenic lipid profile. Similar improvements in CRI have been documented with *Mentha*, clove and citrus peel extracts, where reductions in LDL-C and TG alongside modest HDL increases contribute to restoring cardiovascular balance. Mechanistically, reductions in intestinal lipid absorption (tannins), inhibition of hepatic cholesterol synthesis (citrus and mint flavonoids) and antioxidant stabilization of lipoproteins (clove phenolics) collectively explain the improved CRI in treated groups. The CRI improvements observed align with published findings demonstrating that botanical polyphenols exert multi-target lipid-modifying actions [24].

AI was significantly elevated in HFD-fed rats (10.28), indicating severe dyslipidemia, but decreased substantially following atorvastatin and tea treatments, with Dose 3 (1.27) restoring the index toward near-normal levels. Studies involving citrus peel, peppermint and clove extracts have reported similar reductions in atherogenic indices due to their combined TG- and LDL-lowering effects [24]. The decreased AI in the tea-treated groups reflects the synergistic actions of its phytochemical components: tannins that reduce fat absorption, flavonoids that suppress hepatic lipid synthesis and improve β -oxidation, and antioxidants from mint and cloves that protect lipoproteins from oxidative modification [25, 26]. These mechanisms correspond closely to previously published work, supporting the conclusion that the formulated lime–mint–clove tea effectively improves atherosclerotic status in HFD-induced obese rats.

The serum creatinine concentrations showed a marked elevation in the HFD control group (68.57 ± 8.01 mg/L), confirming HFD-induced renal strain, whereas all tea-treated groups exhibited significantly lower values (Tea-75 mg/kg: 42.86 ± 8.02 mg/L; Tea-150 mg/kg: 30.00 ± 5.25 mg/L; Tea-300 mg/kg: 28.29 ± 3.83 mg/L), approaching or matching the normal control (26.33 ± 3.13 mg/L) and atorvastatin (26.78 ± 6.34 mg/L). This indicates no nephrotoxic effect of the tea and suggests a protective influence at higher doses. Similarly, hepatic enzyme markers ALAT and ASAT, which rise in response to hepatocellular injury, were substantially elevated in the HFD control group (ALAT: 29.40 ± 5.72 U/L; ASAT: 31.15 ± 5.99 U/L) but remained within normal ranges for tea-treated rats, particularly at 150 mg/kg and 300 mg/kg doses (e.g., ALAT: 22.05 ± 5.88 U/L and 29.75 ± 4.46 U/L; ASAT: 11.38 ± 2.63 U/L and 14.70 ± 1.99 U/L). These patterns align with previous findings that plant-derived polyphenols mitigate HFD-induced hepatic stress [27, 28]. Mechanistically, flavonoids such as hesperidin, naringin, and rosmarinic acid exert hepatoprotection by enhancing antioxidant enzyme expression (SOD, CAT, GPx), suppressing NF- κ B-mediated inflammation, and reducing lipid peroxidation [29]. Tannins additionally inhibit digestive lipases, bind pro-oxidant metals, and scavenge reactive oxygen species, thereby decreasing oxidative hepatic burden. Eugenol from cloves prevents membrane damage by stabilizing hepatocyte lipid bilayers [30]. Altogether, the creatinine, ALAT, and ASAT profiles confirm that the tea formulation is non-toxic at all tested doses and demonstrates a dose-dependent protective effect against HFD-induced hepatic and renal impairment, consistent with established findings in obesity models.

Conclusion

The three formulations of lime–mint–clove (LMC) tea (L1, L2, and L3) showed similar values for total phenolic content, flavonoids, tannins, moisture, vitamin C, and crude fat, indicating that variations in ingredient ratios did not significantly affect their phytochemical composition. The notably high tannin content, greater than that of many traditional teas, suggests a strong astringent taste and high antioxidant potential. Low moisture content also indicates good storage stability, while the presence of vitamin C adds nutritional value despite processing losses.

Biological evaluation demonstrated that LMC tea produced significant hypolipidemic, hepatoprotective, and nephroprotective effects in obese rats, with the 150 mg/kg dose showing the most favorable outcomes. These effects likely result from the synergistic actions of polyphenols, flavonoids, and tannins that enhance antioxidant activity and lipid metabolism, then reducing ALAT, was associated with a significant increase in creatinine levels, suggesting lack of side effects during treatment in obese male rats, highlighting its potential as a functional food and natural alternative in obesity management.

Overall, the study supports the potential of LMC tea as a natural anti-obesity agent capable of improving lipid balance, reducing oxidative stress, controlling weight gain in obesity and show the lack of side effects during toxicology analysis and observation. Further studies are recommended to clarify the molecular mechanisms, confirm long-term safety, then study the histopathological analysis, and explore its possible use in human obesity management.

Conflict of Interests

The authors declare no conflict of interest.

References

1. Sirwan Khalid Ahmed, Ribwar Arsalan Mohammed (2025) Obesity: Prevalence, causes, consequences, management, preventive strategies and future research directions.
2. Larissa Pone Simo, Valirie Ndip Agbor, Francine Zeuga Temgoua, Leo Cedric Fosso Fozeu, Divine Tim Bonghaseh, et al. (2021) Prevalence and factors associated with overweight and obesity in selected health areas in a rural health district in Cameroon: a cross-sectional analysis. *Public Health*. 21: 475.
3. Lei X, Rezaei MJ (2025) Synergistic effects of polyphenols and exercise on obesity:targeting metabolism, muscle function, and adipose tissue remodeling.
4. Fakhri LA, Ghanbarzadeh B, Falcone PM (2023) Development of a Novel Low-Calorie, Lime Juice-Based Prebiotic Beverage Using a Combined Design Optimization Methodology. *Foods*.12: 680.
5. Siyu Liua, Shiming Lib, Chi-Tang Hoa (2022) Dietary bioactives and essential oils of lemon and lime fruits. S.Y. Liu et al. / *Food Science and Human Wellness*, 11.
6. Chung-Hsiung Huang, Shun-Yuan Hsiao, Yung-Hsiang Lin, Guo-Jane Tsai (2022) Effects of Fermented Citrus Peel on Ameliorating Obesity in High-Fat Diet Rats. *Molecules*. 27: 8966.
7. Bruno P Chumpitazi, Gregory Kearns, Robert J (2018) Shulman. Review article: The physiologic effects and safety of Peppermint Oil and its efficacy in irritable bowel syndrome and other functional disorders. *Aliment Pharmacol Ther*. 47: 738-52.
8. Behnaz Abiri, Shirin Amini, Mahdi Hejazi, Farhad Hosseinpahah, Afshin Zarghi, et al. (2023) Tea's anti-obesity properties, cardiometabolic health-promoting potentials, bioactive compounds, and adverse effects: A review focusing on white and green teas.
9. Kithinji D, Kasilo OMJ, Kunle O, Doo-Kingue P, Ismail M, et al. (2025) Steps taken by the World Health Organization African Region Member States to standardise herbal medicines: a literature review. *J Glob Health*. 15: 04265.
10. Ángel Calín-Sánchez, Leontina Lipan, Marina Cano-Lamadrid, Abdolreza Kharaghani, Klaudia Masztalerz, et al. (2020) Comparison of Traditional and Novel Drying Techniques and Its Effect on Quality of Fruits, Vegetables and Aromatic Herbs. 9: 1261.
11. Pramod P, Aradwad, Arun Kumar T V, Sahoo PK, Indra Mani, et al. (2021) Key issues and challenges in spice grinding. *Cleaner Engineering and Technology* 5.
12. Jaadan Hayat, Mustapha Akodad, Abdelmajid Moumen, Mourad Baghour, Ali Skalli, et al. (2020) Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of *Marrubium vulgare* L. from 2 different localities of Northeast of Morocco.
13. Roy N, Sharma N, Mohite AM (2025) Evaluating the Impact of Various Drying Processes on the Comprehensive Properties of Thyme Powder (*Thymus vulgaris*) for Retention of Its Bioactive Properties. 7: 59.
14. Mache Andre Gilles, Njouonkou Andre-Ledoux, Carl Moses F Mbofung (2020) Effects of Khaya Tea on Reduction of Obesity and Some Biochemical Parameters of Obese Rats. *J Nutr Obes* 2: 103.

15. André Gilles MACHE, Valentin Désiré GUIAMA, Carl Moses F Mbofung (2015) Anti-Hyperlipidemic And Anti-Weight Gain Effects Of Khaya Tea On High-Fat-Diet Rats. International Journal of Innovation and Scientific Research. 224-31.
16. Rajendra Mani Badala, Divya Badalb, Pourush Badalc, Ashish Kharec, Vinod Kumar, et al. (2011) Pharmacological Action of *Mentha piperita* on Lipid Profile in Fructose-Fed Rats. Iranian Journal of Pharmaceutical Research, 10: 843-8.
17. Adegbola MV, Anyim G, Ntwasa M, Ayeleso AO, Oyedepo TA, et al. (2022) Potential Effect of *Syzygium aromaticum* (Cloves) Extract on Serum Antioxidant Status and Lipid Profiles in Wistar Rats with Artesunate Toxicity. 12: 8216.
18. Seyd-Hosein Abtahi-Eivari, Majid Shokohi, Mohammad Ghorbani, Monireh Halimi, Hossein Hajizadeh, et al. (2021) Effects of Hydroalcoholic Extracts of Cloves (*Syzygium aromaticum*) on the Serum Biomarkers, Antioxidant Status, and Histopathological Changes of Kidneys in Diabetic Rats. Crescent Journal of Medical and Biological Sciences.
19. Yanni Pan, Jingyu Tan, Xingyao Long, Ruokun Yi , Xin Zhao, et al. (2022) Anti-obesity effect of fermented lemon peel on high-fat diet-induced obese mice by modulating the inflammatory response. J Food Biochem. 46: e14200.
20. Huang CH, Hsiao S Y (2022) Effects of Fermented Citrus Peel. Ameliorating Obesity in Rats Fed with High-Fat Diet. Molecules, 27: 8966.
21. Behzad Mesbahzadeh, Mohsen Akbari, Nasroallah Moradi kor, Jalal Bayati Zadeh (2015) The effects of different levels of peppermint alcoholic extract on body-weight gain and blood biochemical parameters of adult male Wistar rats. Electronic Physician.
22. Parichehr Yaghmaie, Kazem Parivar, Minou Haftavar (2011) Effects of *Citrus aurantifolia* peel essential oil on serum cholesterol levels in Wistar rats. Journal of Paramedical Sciences (JPS).
23. Tsutomu Hirano (2025) Excess Triglycerides in Very Low-Density Lipoprotein (VLDL) Estimated from VLDL-Cholesterol could be a Useful Biomarker of Metabolic Dysfunction Associated Steatotic Liver Disease in Patients with Type 2 Diabetes. J Atheroscler Thromb, 32: 253-64.
24. Basma S Ismail, Basant Mahmoud, Eman S Abdel-Reheim, Hanan A Soliman, Tarek M Ali, et al. (2022) Atherosclerosis Induced by High-Fat Diet via Modulation of Hyperlipidemia. Oxidative Stress, and Inflammation Oxidative Medicine and Cellular Longevity.
25. Shi-Yu Cao, Cai-Ning Zhao, Ren-You Gan , Xiao-Yu Xu , Xin-Lin Wei, et al. (2019) Effects and Mechanisms of Tea and Its Bioactive Compounds for the Prevention and Treatment of Cardiovascular Diseases: An Updated Review. Antioxidants. 8: 166.
26. Liu Y, Liu C, Kou X, Wang Y, Yu Y, et al. (2022) Synergistic Hypolipidemic Effects and Mechanisms of Phytochemicals: A Review. Foods. 11: 2774.
27. Joost M Lambooij, Vivien Chavanelle, Marie Vallier, Hendrik JP, van der Zande, et al. (2025) The polyphenol-rich plant extract Totum-448 decreases hepatic steatosis and inflammation in diet-induced MASLD mice Check for updates. npj Gut and Liver. 2: 20.
28. Han Y, Chen Z (2025) Effect of dietary polyphenols along with exercise on hepatic transcriptional regulators of lipid metabolism, Front Nutr. 12: 1531327.

29. Yue-hong Lu, Yue Hong, Tian-yang Zhang, You-xia Chen, Zhao-jun, et al. (2022) Rosmarinic acid exerts anti-inflammatory effect and relieves oxidative stress via Nrf2 activation in carbon tetrachloride-induced liver damage. *Nutrition Research* 2022, 66: 8359.

30. Shakir Ali, Ram Prasad, Amena Mahmood, Indusmita Routray, Tijjani Salihu Shinkafi, et al. (2014) Eugenol-rich Fraction of *Syzygium aromaticum* (Clove) Reverses Biochemical and Histopathological Changes in Liver Cirrhosis and Inhibits Hepatic Cell Proliferation. *Journal of Cancer Prevention*. 19: 288-300.

31. Neeraj Kumar Saini, Manmohan Singhal (2012) Anti-inflammatory, analgesic and antipyretic activity of methanolic *Tecoma capensis* leaves extract. *Asian Pacific Journal of Tropical Biomedicine*. All rights