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Original Research Article

Combination Effect of Slow Interval Training and Lemongrass Ethanol Extract (*Cymbopogon citratus*) on Body Weight and Fat Mass in Male Wistar Rats (*Rattus norvegicus* Sp) Obesity Model

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Abstract

Background: Obesity, a global health crisis affecting over 890 million adults, requiring effective and safe management strategies. Lifestyle-based interventions, such as physical exercise and herbal supplementation, provide promising non-invasive alternatives. Slow Interval Training (SIT) improves fat metabolism with low injury risk, while lemongrass (*Cymbopogon citratus*) contains bioactive compounds with anti-obesity potential.

Objective: This study investigates the combined effects of SIT and lemongrass ethanol extract on body weight and fat mass in a high-fat diet (HFD)-induced obese male Wistar rat model.

Methods: Thirty-five male Wistar rats were divided into five groups: healthy control, obese control, SIT only, lemongrass extract only, and SIT + lemongrass. Obesity was induced through a high-fat diet before intervention. Body weight was measured weekly, and fat mass was analyzed after eight weeks of treatment.

Results: Obese controls showed significant weight gain ($p<0.001$). The lemongrass-only group showed a 12.7% weight reduction, while the combination group achieved the lowest final weight (200 g) and the lowest fat mass (2.39 g, $p=0.048$). Significant intergroup differences appeared from week 1 and persisted until week 13 ($p<0.05$). The combination group maintained stable weight after intervention, outperforming SIT or lemongrass alone.

Conclusion: The combination of SIT and lemongrass ethanol extract significantly reduces body weight and fat mass in obese rats, demonstrating synergistic efficacy. Lemongrass inhibits fat absorption and enhances metabolic regulation, while SIT promotes fat oxidation. This dual approach offers a viable, non-invasive strategy for obesity management, warranting further clinical exploration.

Keywords: *Obesity; Slow interval training; Lemongrass; Wistar rats; Body weight; Fat mass.*
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INTRODUCTION

Obesity is a global public health issue with a rising prevalence over the past four decades. As of 2016, 39% of adults worldwide were overweight and 13% were obese.[1] By 2022, approximately 16% of adults globally were overweight, amounting to over 890 million obese individuals or 43% of the global adult population. In children under 5 years old, 37 million were reported overweight. Regional differences in prevalence range from 31% in Southeast Asia and Africa to 67% in the Americas.[2] In Indonesia, the 2018 Basic Health Research Survey noted that adult obesity doubled from

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10.5% in 2007 to 21.8% in 2018. Ministry of Health data from 2023 indicates this rate remains around 21%, with a higher prevalence in women.[3]

Non-communicable diseases like cancer, fatty liver, respiratory conditions, kidney disease, cardiovascular disease, type 2 diabetes, dyslipidemia, and musculoskeletal disorders are all made more likely by obesity.[4] Body weight is often used as a representative marker for obesity because it is the core component in calculating body mass index (BMI), which is the most common method in clinical and public health settings. Obesity is classified into categories: class I (BMI 30-35 kg/m^2), class II (BMI 35-40 kg/m^2), class III or morbid obesity (BMI ≥40 kg/m^2). Additional measurements include waist circumference (≥90 cm in men, ≥80 cm in women) and waist-to-hip ratio (≥0.9 in men, ≥0.85 in women), particularly for Asian populations.[5,6] Body weight remains a widely used obesity indicator due to its simplicity, affordability, and standardization. It correlates with health risks and is suitable for population-based assessments. A higher BMI typically reflects increased body fat and a greater risk for conditions such as heart disease, diabetes, and hypertension. Although not ideal for individual diagnosis, BMI is practical for tracking trends and informing health policy. Organizations like the WHO use BMI as the standard for defining overweight and obesity.[7,8]

However, body weight and BMI have limitations as they do not differentiate between fat, muscle, and bone mass. They also do not measure fat distribution or percentage directly. In contrast, body fat mass—especially visceral fat—is more indicative of obesity-related health risks. Studies show that body fat percentage is more strongly linked to conditions like metabolic syndrome and cardiovascular disease. Measuring fat mass allows for a direct assessment of the most relevant component of obesity, offering better predictive value than BMI alone.[9–11] Given the persistently high prevalence and health burden of obesity, there is an urgent need for accessible, safe, and sustainable interventions beyond conventional pharmacological and surgical options. This has increased global interest in lifestyle-based approaches, including structured exercise programs and plant-derived supplementation, which may offer complementary and cost-effective solutions for weight management.

Obesity management conventionally involves pharmacological or surgical approaches, but these methods often provide short-term results, may cause side effects, and are associated with weight regain post-treatment.[1] Lifestyle modifications remain the cornerstone of obesity management, especially dietary control and physical activity.[12] One effective and safer physical activity method is Slow Interval Training (SIT), which improves metabolism and enhances fat oxidation while being well-tolerated by obese individuals.[13] Combining exercise with appropriate dietary intake, especially natural ingredients, yields optimal outcomes. Herbal supplementation is increasingly recognized for its potential in obesity treatment.[14,15] Various medicinal plants are used for managing diseases such as diabetes, cancer, and cardiovascular conditions, including obesity.[16] One such plant is lemongrass (*Cymbopogon citratus*), which contains bioactive compounds like citral, geraniol, and flavonoids known for their anti-obesity properties.[17,18] Previous studies have shown that lemongrass extract at a dose of 300 mg/kgBW in obese mice reduced BMI and fasting blood glucose, and improved glycemic control.[19] Another study combining 500 mL daily lemongrass tea intake with 45 minutes of moderate-intensity physical activity showed positive results in reducing obesity risk factors.[20]

Based on this evidence, the present study is the first to investigate the combined effect of Slow Interval Training (SIT) and lemongrass ethanol extract on body weight and fat mass in obese male Wistar rats. This research aims to evaluate whether combining SIT and lemongrass provides a synergistic, non-invasive approach to obesity management and contributes to developing novel lifestyle-based interventions.

MATERIALS AND METHODS

Research Design

Post-test with control group design study on obesity model mice with high-fat diet. The study was conducted in February-June 2025 at the Pharmacology and Therapeutics Laboratory, Department of Pharmacology, Faculty of Medicine, Universitas Sumatera Utara. This study has been approved by the Research Ethics Committee of the University of North Sumatra. This study was approved by the Animal Research Ethics Committee (AREC) of the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, with Ethical Clearance Number No. 0064/KEPH-FMIPA/2025. All procedures involving animals were conducted in accordance with ethical standards and institutional guidelines for animal research.

Sample

White male Wistar rats (*Rattus norvegicus* Sp.) from the University of North Sumatra's Faculty of Medicine Laboratory were used in this study. The use of the Federer formula obtained a total of 30 rats in 5 groups.[21] The rats were randomized according to the inclusion and exclusion criteria determined by the researcher. The inclusion criteria included male Wistar rats aged 6-8 weeks with a body weight of 100-150 grams, healthy, and appeared active during the study. The exclusion criteria were rats that experienced other diseases or injuries during the study and rats that could not survive until the end of the study. The rats were placed in a cage made of plastic (20 x 30 x 30 cm). The cage was covered with wire gauze, the base of the cage was lined with rice husks at a thickness of 0.5-1 cm and replaced every day. In 1 cage containing 4 rats, the room light was controlled 12 hours of light and 12 hours of darkness at a temperature of 22-25ºC. Mice were given standard feed consisting of 12% fat, 60% carbohydrates, and 28% protein and were given ad libitum water.

Obesity Model

Obesity induction was performed by administering a high-fat–high-carbohydrate (HFHC) diet for 5 weeks.

The diet provided 41% of total energy from fat, 41% from carbohydrates, and 18% from protein, consistent with the macronutrient composition commonly used in diet-induced obesity (DIO) models.[22,23] Fat sources were primarily *lard* and *soybean oil*, carbohydrates were derived from *sucrose* and *cornstarch*, and protein from *casein*. The diet composition was adapted from commercially available formulations (e.g., D12079B, Research Diets Inc.) to match the macronutrient content reported in similar studies.[22] The success of the obesity model was confirmed by a body-weight gain exceeding 20% of the initial weight following the 5-week intervention period.[23]

Intervention

A total of 35 rats were divided into 5 groups (K1, K2, P1, P2, P3). Group K1 were healthy rats with standard diet, K2 were obese rats without intervention, P1 were obese rats with slow interval training intervention, P2 were obese rats with lemongrass ethanol extract intervention at a dose of 300 mg/kgBW, and P3 were obese rats with a combination of slow interval training and lemongrass ethanol extract at a dose of 300 mg/kgBW. All rats were acclimatized for 7 days followed by induction of the obesity model for 5 weeks. After 5 weeks, weight gain was examined. The intervention was given for 8 weeks with body weight weighed once a week. After the study, all experimental animals were terminated with intramuscular ketamine sedation at 30 mg/kgBW followed by abdominal fat weight collection and measurement.

Slow Interval Training

Obese model mice ran on a treadmill at a speed of 20 m/min for 10 sessions, each lasting 2 minutes with 1 minute of active rest in between (20 m/min, 10x2 minutes, 1 minute rest). During the 8 weeks of intervention, slow interval training was done.

Lemongrass (Cymbopogon citratus)

Lemongrass leaf sampling was carried out at one location. Lemongrass was washed clean, sorted and dried in a drying cabinet using a lamp until dry. Lemongrass is said to be dry if the lemongrass is squeezed with fingers and the leaves will be crushed. The dried lemongrass was ground into powder with a blender. Amount of 80 grams of kitchen lemongrass simplicia was macerated with 2 liters of 96% ethanol solvent in a large jar for 2-3 days with stirring every 24 hours. Furthermore, from the filtered maceration results, filtrate was obtained, extraction was carried out three times. Then concentrated with a rotary evaporator at a temperature of 50°C until a crude extract was obtained. Phytochemical screening was carried out on the extract obtained to determine the metabolite compounds contained in the ethanol extract of lemongrass leaves (*Cymbopogon citratus*) which have the potential as antiobesity. Administration of lemongrass extract was carried out using a gavage needle at a dose of 300 mg/kgBW 1x/day for 8 weeks.

Statistical Analysis

Univariate descriptive analysis was determined. Normality test using Shapiro-Wilk test. Normally distributed data will be continued with parametric one-way ANOVA test followed by post-hoc test if significant. Non-normally distributed data will be continued with non-parametric test with Kruskall-Wallis test. The mean difference test before and after HFD administration was performed using parametric pair T test or non-parametric Wilcoxon test.

RESULTS

Phytochemical screening of lemongrass ethanol extract (*Cymbopogon citratus*) showed the presence of several bioactive compounds. The extract was positive for saponins (foam formation lasting more than one second), tannins (black coloration with $FeCl_3$ test), flavonoids (red layer formed above the amyl alcohol layer), phenolic acids (blue–black coloration with $FeCl_3$ reagent), alkaloids (weak orange precipitation), steroids (green to red coloration), and terpenoids (reddish-brown ring formed at the interface in the Salkowski test). These findings are summarized in table 1.

Weight graph in each group for 14 weeks show groups P1, P2, and P3 showed gradual weight loss with the lowest weight loss by group P2 (Figure 1). The results of repeated measures ANOVA on the weight variable in K1 and K2 showed $P<0.001$, while in P1, P2, and P3 showed $P>0.05$. Waist circumference data graph in each group for 14 weeks show fluctuative result with gradual decrease found in the group P1 dan P3, yet the lowest data reached by group P2 (Figure 2). The results of repeated measures ANOVA on the waist circumference variable in all groups showed $P<0.001$. Height data graph in each group for 14 weeks show fluctuative result (Figure 3). The results of Repeated Measures ANOVA on the height variable in all groups showed $P<0.001$. On fat mass parameter, the lightest to heaviest mass were found sequentially in groups P3, P2, K1, P1, and K2 with significant baseline results at week 13. These findings are summarized in Table 2.

DISCUSSION

Obesity in this study was achieved through HFD administration. High-fat diets cause a caloric surplus due to their dense energy, leading to excessive intake compared to expenditure. Excess energy is stored as triglycerides in adipose tissue, which triggers adipocyte

Table 1. Results of phytochemical screening of lemongrass ethanol extract (*Cymbopogon citratus*)

Sample Test	Hasil +/-	Description
Saponin	+	Foam is formed for more than a second
Tannin	+/Galat	Formed in black
Flavonoid	+	A clear red layer is formed above the amyl alcohol layer
Phenolic acids	+	Formed in blue–black coloration with $FeCl_3$ reagent
Alkaloid	±	Weak orange precipitation
Steroid	+	Formed in green and red
terpenoid	+	Reddish-brown ring formed at the interface

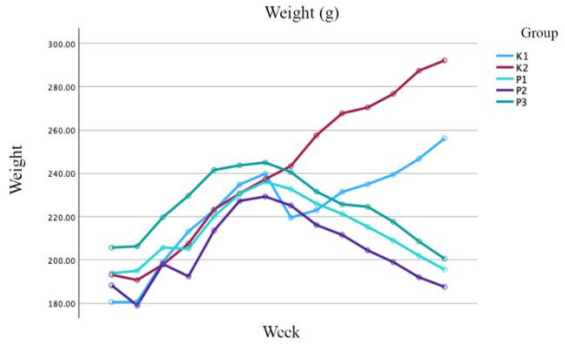

Figure 1. Body weight changes (g) of male Wistar rats in groups K1, K2, P1, P2, and P3 over 14 weeks of intervention.

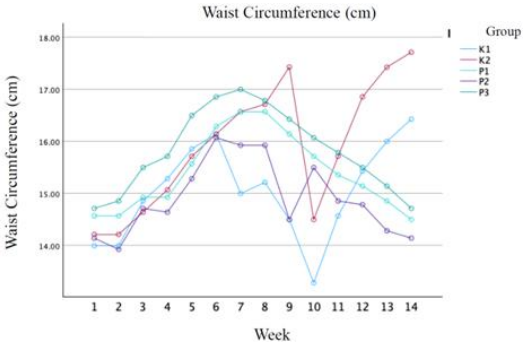

Figure 2. Waist circumference changes (cm) of male Wistar rats in groups K1, K2, P1, P2, and P3 over 14 weeks of intervention.

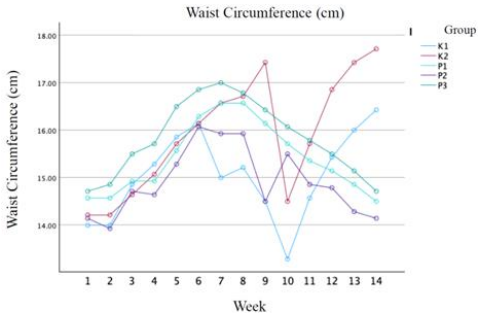

Figure 3. Height changes (cm) of male Wistar rats in groups K1, K2, P1, P2, and P3 over 14 weeks of intervention.

hypertrophy and hyperplasia. These changes impair adipose tissue function, increasing pro-inflammatory adipokines and reducing anti-inflammatory adiponectin.[24]

When subcutaneous adipose tissue reaches its storage capacity, lipids accumulate in visceral adipose tissue (VAT), liver, muscle, and other organs. This ectopic fat deposition contributes to insulin resistance and metabolic dysfunction. Diets high in saturated fat further promote overeating by altering brain–gut signaling and reducing leptin sensitivity. As a result, experimental animals develop hyperphagia and accelerated weight gain.[25]

The type of fat in HFD is also important. Diets rich in saturated fats (e.g., lard, palm oil) are more strongly associated with inflammation and insulin resistance compared to unsaturated fats, which have milder metabolic effects and may even improve lipid profiles. Saturated fats promote overeating by altering brain-gut signaling and reducing leptin sensitivity. This creates a cycle of increased food intake and fat storage, resulting in hyperphagia in experimental animals, which further accelerates weight gain.[26,27]

In the long term, HFD induces hormonal and metabolic dysfunctions, including leptin resistance, insulin resistance, and mitochondrial dysfunction. Leptin's ability to suppress appetite and increase energy expenditure becomes impaired, perpetuating overeating. Saturated fats disrupt insulin signaling by altering cell membrane composition, reducing GLUT4 translocation, and increasing inflammatory cytokine release. This reduces glucose uptake and promotes hyperglycemia.[27]

Excessive lipid accumulation also uncouples β-oxidation from the tricarboxylic acid (TCA) cycle. This increases reactive oxygen species (ROS) and decreases fatty acid oxidation efficiency. Consequently, obese adipose tissue recruits pro-inflammatory M1 macrophages that maintain chronic low-grade inflammation. Circulating free fatty acids (FFAs) activate Toll-like receptors on immune cells, stimulating NF-κB and JNK pathways that exacerbate insulin resistance. In addition, HFD increases intestinal permeability, allowing lipopolysaccharides (LPS) from

Table 2. Results of observations of body weight, height, waist circumference, and fat mass parameters

Variable	Group (Mean ± SD) (n=35)				
	K1 (n=7)	K2 (n=7)	P1 (n=7)	P2 (n=7)	P3 (n=7)
Weight (g)					
Baseline	180.57±18.97	193.14 ± 11.88	193.85 ± 26.11	188.28±14.43	205.71±13d.84
Week-1	180.57 ±17.9	190.71±13.38	195.0±23.1	179.0±13.19 [c]	206.28±17.17[ad]
Week-2	199.14±21.9	197.85±15.47	205.71±20.63	198.14±9.51	219.71±17.15
Week-4	223±26.65	223.28±17.14	220.14±20.27	213.57±9.28	241.57±26.61
Week-5					
P					
Week-8	223±22.56	257.57±14.66	226.0±19.79 [b]	216.14±14.69 [b]	231.57±27.76 [bd]
Week-12	246.71±33.45	287.42±16.97	202.0±16.02 [ab]	192.0±12.68 [ab]	208.57±15.81 [abd]
Week 13	256.14±34.91	292.14±19.27	195.57±15.08	187.57±11.17	200.57±14.86
P	0.002[#]	<0.001[#]	0.791	0.905	0.381
Waist circumference (cm)					
Baseline	14.0±0.65	14.21±0.56	14.57±0.93	14.14±0.62	14.71±0.48
Week-1	14.0±0.64	14.21±0.56	14.57±0.78	13.92±0.53	14.85±0.69
Week-2	14.85±1.46	14.64±0.8	14.92±0.73	14.71±0.39	15.5±0.81
Week-4	15.85±1.21	15.71±0.85	15.57±0.83	15.28±0.39	16.5±1.29
Week-8	14.5±1.11	17.42±0.83 [a]	16.14±0.8 [ab]	14.5±1.11[ab]	16.42±1.36 [ad]
Week-12	16±1.52	17.42±1.13	14.85±0.55 [ab]	14.28±0.39 [ab]	15.14±0.74 [ab]
Week-13	16.42±0.95	17.71±0.75	14.5±0.5[b]	14.14±0.37	14.71±0.63
P	0.001[#]	<0.001[#]	0.766	1.000	1.000
Height (cm)					
Baseline	19.14±1.06	19.57±0.78	19.71±0.48	19.71±0.48	19.28±0.75
Week-1	20.57±0.53	19.57±078	19.85±0.37	20±0.57	20.5±0.53
Week-2	20.85±0.37	20.57±0.53	20.85±0.37	20.14±0.37	20.85±0.37
Week-4	21.14±0.62	21.07±0.18	21.14±0.89	21.07±0.45	21.57±0.45
Week-8	22.42±0.53	21.92±0.18	22.14±0.89	22±0.57	21.71±0.75
Week-12	22.57±0.53	22.42±0.53	22.57±0.78	22.14±0.69	22.42±0.78
Week-13	22.71±0.75	22 .42±0.53	22.57±0.78	22.14±0.69	22.14±0.69
P	<0.001[#]	<0.001[#]	<0.001[#]	<0.001[#]	<0.001[#]
Fat Mass (g)					
Week-13	2.65±0.94	3.88±0.55	2.96±1.14	2.56±1.18	2.39±0.80

[*]One-way ANOVA test is significant at p<0.05; [#]Paired T-Test Baseline vs Week-13 is significant at p<0.05; [a]significant in the LSD post-hoc test against K1; [b]significant in the LSD post-hoc test against K2; [c]significant in the LSD post-hoc test against P1; [d]significant in the LSD post-hoc test against P2.

gut microbiota to enter circulation and further drive systemic inflammation.[28]

Previous studies support these findings. In rat models, unsaturated HFDs given for 11–24 weeks induced a 31% increase in body weight and impaired glucose tolerance (AUC: 171% of control). In contrast, saturated HFDs given for 20 weeks led to cardiomyocyte hypertrophy, interstitial fibrosis, and hyperglycemia.[29–31]

Slow interval training (SIT) alternates low-intensity exercise with rest periods, maintaining the body in a fat-oxidation zone for extended durations. At lower intensities, fat is used as the primary fuel source compared to carbohydrates. This directly promotes fat utilization and spares glycogen stores. SIT stimulates mitochondrial biogenesis in muscle cells, improves oxygen delivery, and enhances fatty acid oxidation. These adaptations help lower lactic acid accumulation and improve glucose uptake, ultimately reducing insulin resistance and fat storage. SIT also modulates key metabolic hormones, increasing adiponectin (fat-burning) and improving leptin sensitivity.[32–34]

Another advantage of SIT is its lower joint stress compared to high-intensity interval training (HIIT), making it sustainable for obese or sedentary individuals. Although SIT has a smaller excess post-exercise oxygen consumption (EPOC) compared to HIIT, its longer duration and repeatability create a cumulative caloric deficit critical for weight loss. Studies have shown that SIT performed at 40–60% HRmax for 30–45 minutes, three times per week for 6–12 weeks, effectively reduces body weight and improves insulin sensitivity with higher adherence rates than HIIT. SIT also significantly increases PGC-1α expression, enhancing basal metabolism and fat-burning capacity.[35–37]

Phytochemical screening of lemongrass ethanol extract (*Cymbopogon citratus*) demonstrated the presence of several secondary metabolites, including saponins, tannins, flavonoids, phenolic acids, alkaloids (weakly positive), steroids, and terpenoids (Table 1). Among these, citral, flavonoids, and phenolic acids are considered the major bioactive compounds contributing to the anti-obesity effects of lemongrass. Citral modulates lipid metabolism through activation of the PPARγ/AMPK pathway, while flavonoids act as potent antioxidants and improve insulin sensitivity. Phenolic acids further enhance metabolic regulation by

suppressing lipogenesis and promoting glucose homeostasis. Other compounds such as terpenoids, tannins, and saponins may provide supportive roles in fat oxidation, enzyme inhibition, and overall metabolic balance.[18,38–41]

Although the present study performed only qualitative phytochemical screening, previous quantitative analyses of Cymbopogon citratus extracts indicate substantial levels of phenolic and flavonoid compounds. Reported total phenolic content (TPC) for lemongrass extracts varies widely with extraction method and solvent, with values ranging approximately from ~5 to >60 mg GAE/g extract, while total flavonoid content (TFC) has been reported in ranges from ~2 up to >50 mg QE/g (or equivalently 20–50 mg QE/100 g) in some extraction conditions. These literature values support the qualitative detection of phenolic acids and flavonoids in the current ethanol extract and suggest that these compounds may contribute significantly to the extract's antioxidant and metabolic effects. Future work should include direct quantification of TPC and TFC (e.g., Folin–Ciocalteu and aluminum chloride assays) for the specific extract used in this study to better correlate phytochemical content with biological outcomes.[42–44]

Animal studies have reported that ethanolic lemongrass extract (200 mg/kgBW) reduced body weight by 22%, improved insulin sensitivity, and decreased leptin and C-reactive protein (CRP) levels. Aqueous extract (250 mg/kgBW) reduced BMI by 15% and enhanced fat oxidation in metabolic syndrome models. Lemongrass essential oil (300 mg/kgBW) caused 28% visceral fat loss and normalized lipid profile in hyperlipidemic rats.[45] Higher aqueous extract doses (600 mg/kgBW) showed appetite suppression and 18% weight reduction in Wistar rats, while isolated citral (20 mg/kgBW) increased metabolic rate and reduced adipocyte size.[40,45,46]

Both SIT and lemongrass independently reduce body weight and fat mass, but their combination may provide synergistic benefits. Lemongrass reduces fat absorption and modulates adipokines, whereas SIT enhances fat oxidation and improves insulin sensitivity. Both interventions also reduce inflammation—lemongrass via antioxidant effects and SIT via lowering CRP—potentially offering additive benefits for metabolic health.[47–49] The strength of this study lies in its novelty, being among the first to investigate the combined effects of SIT and lemongrass on diet-induced obesity. The limitation of this study is the reliance solely on body weight and fat mass parameters without biochemical markers such as leptin or CRP, which would provide deeper mechanistic insight.

CONCLUSION

Combination of Slow Interval Training (SIT) and lemongrass (*Cymbopogon citratus*) ethanol extract are effective on improving body weight and fat mass in male Wistar rats (*Rattus norvegicus* Sp) obesity model.

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