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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

Sulyaprilawati Battri Siahaan¹, Yetty Machrina^{2,5*}, Tri Widyawati³, Rusdiana⁴, Maya Savira⁵

¹ Master Program in Biomedical Sciences, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

^{2.5} Department of Physiology, Faculty of Medicine, Universitas Sumatera Utara, Indonesia
³ Departement of Pharmacology and Therapy, Faculty of Medicine, Universitas Sumatera Utara, Indonesia

⁴Department of Biochemistry, Faculty of Medicine, Universitas Sumatera Utara, Indonesia

*Corresponding author:

Name: Yetty machrina

Phone Number: +62 81397382797 Email address: yetty@usu.ac.id

Running title: Combination Training and Lemongrass on Obesity

Abstract

Background: Obesity, a global health crisis affecting over 890 million adults, demands effective interventions beyond conventional pharmacological and surgical approaches. Lifestyle modifications, including physical exercise and herbal supplementation, offer promising alternatives. Slow Interval Training (SIT) enhances fat metabolism with minimal injury risk, while lemongrass (Cymbopogon citratus) exhibits anti-obesity properties through bioactive compounds like citral and flavonoids. **Djectivity:** This study investigates the combined effects of SIT and lemongrass and 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 controls (K1), obese controls (K2), SIT intervention (P1), lemongrass extract (300 mg/kgBW, P2), and combined SIT + lemongrass (P3). Obesity was induced via a 41% fat diet for five weeks. Interventions were administered over eight weeks, with weekly body weight measurer 22 ts and post-intervention fat mass analysis. Statistical comparisons used one-way ANOVA, LSD post-hoc tests, and paired T-tests (p<0.05 significance). Result: Weight Changes on K2 (obese controls) showed significant weight gain (193.14±11.88g to 292.14±19.27g, p<0.001). On P2 (lemongrass) and P3 (combination) achieved the lowest final weights (187.57±11.17g and 200.57±14.86g, respectively), with P2 showing a 12.7% reduction from baseline (p=0.905 vs. baseline). Significant intergroup differences emerged at weeks 1 (p=0.038), 8 (p=0.008), and 12-13 (p<0.001). On P3 had the lowest fat mass $(2.39\pm0.80g \text{ vs. } \text{K2}: 3.88\pm0.55g,$ p=0.048). The combination group (P3) maintained stable weight post-intervention, outperforming SIT-alone (P1) and lemongras colone (P2) in sustained fat reduction. 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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42 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. 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. Indonesia, the 2018 Basic Health Research Survey noted that adult obesity doubled from 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.

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 weiglt for often used as a representative marker for obesity because it is the core component in calculating body m 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/m²), class II (BMI 35-40 kg/m²), class II (BMI ≥40 kg/m²), and morbid obesity. 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. 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. 1-9

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. ^{10–12}

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. Lifestyle modifications remain the cornerstone of obesity management, especially dietary control and physical activity. ¹³ 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. ¹⁴ Combining exercise with appropriate dietary intake, especially natural ingredients, yields optimal outcomes. Herbal supplementation is increasingly recognized for its potential in obesity treatment. ^{15,16} Various medicinal plants are used for managing diseases such as diabetes, cancer, and cardio cular conditions, including obesity. ¹⁷ One such plant is lemongrass (*Cymbopogon citratus*), which contains bioactive compounds like citral, geraniol, and flavonoids known for their anti-obesity properties. ^{18,19} 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. ²⁰ 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. ²¹

Based on this evidence, it is important to further explore the combination of Slow Interval Training and lemongrass ethanol extract as a potential obesity intervention. Such a combination could provide complementary, non-invasive, and natural strategy for reducing body weight and fat mass. Therefore, this study aims to determine the combined effect of Slow Interval Training (SIT) and lemongrass (Cymbopogon citratus) ethanol extract on body weight and fat mass in male Wistar rats (Rattus norvegicus Sp) in an obesity model. The results are expected to contribute to the scientific basis for developing effective obesity therapies involving physical activity and herbal supplementation.

MATERIAL AND METHODS

Study Design

Post-test with control group design study on obesity model mice with high-fat diet. Be study was conducted in February-June 2025 at the Pharmacology and Theraportics Laboratory, Department of Pharmacology, Faculty of Medicine, University of North Sumatra. This study has been approved by the Research Ethics Committee of the University of North Sumatra.

Sample

White male Wistar rats (Rattus norvegicus Sp.) from the University of North Sumatra's Faculty of Medicine Laboratory were used in this 11 dy. The use of the Federer formula obtained a total of 30 rats in 5 groups. 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 criteriagerer 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 thick 10 s 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 22-25°C. 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 diet (HFD) for 5 weeks. High fat diet consists of 41% fat, 41% carbohydrates, and 18% protein. The success of the obesity model was assessed by weight gain >20% of the initial body weight after the intervention.

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 intervent n, P1 were obese rats with slow interval training intervention, P2 were obese rats with lemongrass ethanol extract intervention at a dosc 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 50

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

thesptential 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

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. 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. 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. 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 1.

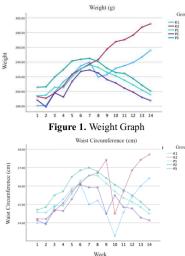


Figure 2. Waist Circumference Graph

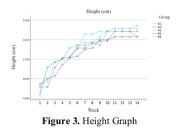


Table 1. Results of observations of body weight, height, waist circumference, and fat mass parameters

*********	11	Gro	up (Mean ± SD) (n=35)		P
Variable	K1 (n=7)	K2 (n=7)	P1 (n=7)	P2 (n=7)	P3 (n=7)	P
Weight (g)						
Baseline	180.57±18.97	193.14 ± 11.88	193.85 ± 26.11	188.28±14.43	205.71±13d.84	0.143
Week-1	180.57 ±17.9	190.71±13.38	195.0±23.1	179.0±13.19°	206.28±17.17ad	0.038*
Week-2	199.14±21.9	197.85±15.47	205.71±20.63	198.14±9.51	219.71±17.15	0.124
Week-4	223±26.65	223.28±17.14	220.14±20.27	213.57±9.28	241.57±26.61	0.173
Week-5	234,85±27,87	230,71±16,36	221,85±44,36	224,71±22,32	247,28±33,75	0.173
P	0,005†	<0,001†	<0,001†	<0,001†	0,003 [†]	
Week-8	223±22.56	257.57±14.66	226.0±19.79 b	216.14±14.69 b	231.57±27.76 bd	0.008*
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	<0.001*
Week 13	256.14±34.91	292.14±19.27	195.57±15.08	187.57±11.17	200.57±14.86	<0.001*
P	0.002#	< 0.001#	0.791	0.905	0.381	
Waist circu	mference (cm)					
Baseline	14.0±0.65	14.21±0.56	14.57±0.93	14.14 ± 0.62	14.71±0.48	0.251
Week-1	14.0±0.64	14.21±0.56	14.57±0.78	13.92±0.53	14.85±0.69	0.059
Week-2	14.85±1.46	14.64±0.8	14.92 ± 0.73	14.71±0.39	15.5±0.81	0.439
Week-4	15.85±1.21	15.71±0.85	15.57±0.83	15.28±0.39	16.5±1.29	0.225
Week-8	14.5±1.11	17.42±0.83 a	16.14±0.8 ab	14.5±1.11ab	16.42±1.36 ad	< 0.001*
Week-12	16±1.52	17.42±1.13	14.85±0.55 ab	14.28±0.39 ab	15.14±0.74 ab	<0.001*
Week-13	16.42±0.95	17.71±0.75	14.5±0.5b	14.14±0.37	14.71±0.63	<0.001*
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	0.511
Week-1	20.57±0.53	19.57±078	19.85±0.37	20±0.57	20.5±0.53	0.009*
Week-2	20.85±0.37	20.57±0.53	20.85±0.37	20.14±0.37	20.85±0.37	0.01*
Week-4	21.14±0.62	21.07±0.18	21.14±0.89	21.07±0.45	21.57±0.45	0.451
Week-8	22.42±0.53	21.92±0.18	22.14±0.89	22±0.57	21.71±0.75	0.327
Week-12	22.57±0.53	22.42±0.53	22.57±0.78	22.14±0.69	22.42±0.78	0.758
Weel5 13	22.71±0.75	22 .42±0.53	22.57±0.78	22.14±0.69	22.14±0.69	0.454
P	<0.001#	< 0.001#	< 0.001#	< 0.001 #	<0.001#	
Fat Mass (g	g)					
Week-13	2.65±0.94	3.8 28 .55	2.96±1.14	2.56±1.18	2.39 2 .80	0.048*

*One-way ANOVA test is significant at p<0.05; *Paired T-Test Baseline vs Week-13 is significant at p<0.05; *significant in the LSD post-hoc test against K1; *bsignificant in the LSD post-hoc test against K2; *significant in the LSD post-hoc test against K2; *significant in the LSD post-hoc test against P1; *dsignificant in the LSD post-hoc test against P2.

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 caloric intake compared to expenditure. Excess energy is stored as triglycerides in adipose tissue resulting in adipocyte hypertrophy and

hyperplasia. This impairs adipose tissue function, increasing pro-inflammatory adipokines and decreasing anti-inflammatory adiponectin. When subcutaneous adipose tissue reacles its capacity, lipids accumulate in visceral adipose tissue (VAT), liver, muscle, and other organs, contributing to insulin resistance and metabolic dysfunction. Saturated fat in HFD promotes overeating by altering brain-gut signaling and reducing satiety hormones such as leptin sensitivity. This creates a cycle of increased food intake and fat storage, leading to hyperphagia in patients. ^{22–29}

Long term manifestation will induce hormonal and metabolic dysfunction which lead to leptin resistance, insulin resistance, and mitochondrial dysfunction. Obesity reduced leptin's ability to suppress appetite and increase energy expenditure, perpetuating overeating. Saturated fats impair insulin signaling by altering cell membrane composition, reducing GLUT4 translocation, and increasing inflammatory cytokines. This disrupts glucose uptake and promotes hyperglycemia. Lipid overload uncouples β-oxidation from the tricarboxylic acid (TCA) cycle, increasing reactive oxygen species (ROS) and reducing fatty acid oxidation efficiency. This pathway will lead to systemtic inflammation obese AT recruits pro-inflammatory M1 macrophages, which secrete cytokines that sustain chronic low-grade inflammation. Circulating FFAs from HFDs activate Toll-like receptors on immune cells, triggerite NF-κB and janus kinase (JNK) pathways that exacerbate inflammation and insulin resistance. High fat diet increase intestinal permeability, allowing lipopolysaccharides (LPS) from gut microbiota to enter circulation, further activating systemic inflammation. 22-29 Previous study on rat models given HFD with unsaturated fat for 11-24 weeks show significant weight gain (increase 31%) and impaired glucose tolerance (AUC: 171% of control). Other high saturated fat diet for 20 weeks show cardiomyoctye hypertrophy, interstitial fibrosis, and hyperglicemia. 30-32

Slow interval training alternates low-intensity activity with rest periods, keeping the body in a fat-burning zone for extended durations. At lower intensities, the body relies more on fat as a primary fuel source compared to carbohydrates, promoting direct fat utilization during exercise. This contrasts with high intensity interval training (HIIT), which prioritizes glycogen breakdown due to higher intensity. Slow interval training stimulates the growth of mitochondria in muscle cells, enhancing their capacity to oxidize fatty acids and increasing blood flow to muscles improves oxygen delivery. This pathway supports prolonged fat oxidation and reducing lactic acid buildup thus regular SIT improves glucose uptake in muscles, reducing insulin resistance and fat storage, also modulates hormones like adiponectin (fat-burning) and leptin (appetite regulation), aiding metabolic health. The benefit of slow interval training, SIT's reduced intensity minimizes joint stress, making it sustainable for individuals with obesity or low fitness levels. Consistent energy expenditure, while SIT's post-exercise calorie burn (EPOC) is smaller than HIIT, its longer duration and repeatability create a cumulative caloric deficit critical for fat loss. 33-38

Study showed SIT with an intensity of 40-60% HRmax for 30-45 minutes is effective in reducing body weight and increasing insulin sensitivity in obese individuals with a lower risk of injury compared to HIIT. Recent research proved that SIT performed three times a week for 6-12 weeks is effective in reducing body fat percentage and increasing cardiorespiratory fitness in obese individuals, with a higher level of program compliance compared to high-intensity training protocols. Slow Interval Training significantly increases fat metabolism and PGC-1 α expression in muscle sisue. This expression plays a role in increasing basal metabolism and states that play an important role in metabolic adaptation and fat burning. ³³⁻³⁸

Lemongrass (*Cymbopogon citratus*) exerts anti-obesity effects through bioactive phytochemicals that modulate metabolic pathways. Lemongrass contain diverse bioactive compounds, including citral isomers, flavonoids (luteolin, quercetin, apignin, cynaroside), terpenoids (cymbopogone, cymbopogonol, and β -caryophyllene), phenolic acids (chlorogenic acid, ferulic acid, and p-coumaric acid), and tannins. Citral modulates lipid metabolism, activates peroxisome proliferator-activated receptor gamma/AMP-activated protein kinase (PPAR γ /AMPK)

pathways. Flavonoids act as antioxidant while terpenoid will enhance mitochondrial function and fat oxidation. Phenolic congound will improve insulin sensitivity and suppress lipogenesis. Activation of PPARy induces the browning of white adipose tissue (WAT) into metabolically active beige fat. This process upregulates uncoupling protein 1 (UCP1), enhancing thermogenesis and energy expenditure while AMPK activation will enhances fatty acid oxidation by activating SIRT1-PGC1 α signaling and suppresses lipogenesis in adipocytes by inhibiting mTOR and acetyl-CoA carboxylase (ACC). Inhibiting lipogenesis also via reduce dietary fat absorption by blocking pancreatic lipase activity, limiting triglyceride breakdown. Lemongrass also enhance fat absorption by inhibit α -glucosidase via luteolin derived by flavonoids which slow carbohydrate digestion, lowering postprandial glucose spikes and insulin-driven fat storage. Other mechanism by lemongrass are improved insulin sensitivity by GLUT4 translocation also adipokine modulation by reduces leptin and increase adiponectin. $^{39-44}$

Previous study related administration of ethanolic extract of lemongrass dose 200 mg/kgBB on obese rats show 22% reduction in body weight, improved insulin sensitivity, reduced leptin, and C-reactive protein levels. Then, on aqueous extract dose 250 mg/kgBB, lemongrass administration show 15% BMI reduction and moderate fat oxidation on metabolic syndrome rats. Lemongrass essential oil dose 300 mg/kgBB on hyperlipidaemic rats show 28% visceral fat loss and normalized lipid profile. ⁴⁵ On dose 600 mg/kgBB, aqueous lemongrass leaf extract show appetite suppression and 18% body weight reduction on Wistar rats. ⁴⁶ Isolated citral dose 20 mg/kgBB on diet-induced obese rats show increasing metabolic rate and smaller adipocytes. ^{45,47}

Independently, both SIT and lemongrass show great effectiveness on reducing body weight and fat mass reduction. Hypothesized synergy of SIT and lemongrass are lipid metabolism in which lemongrass contribution on inhibiting fat absorption while SIT will increase fat oxidation during exercise. In adipokine balance, lemongrass will induce adiponectin while decrease leptin and SIT will improve insulin sensitivity. Lemongrass act as antioxidant and SIT will lower CRP, both of them works by reducing inflammation. 48-50 Strengths of this study are the novelty of combining the lemongrass and SIT on obese model. Limitation of this study we only use body weight parameter and fat mass, we did not holistic insight related biochemical markers like leptin or CRP.

CONCLUSIONS

Combination of Slow Interval 44 aining (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.

ETHICAL APPROVAL

This study was approved by the Animal Research Ethics Committee (AREC) of the Faculty of Mathematics and Natural Science. 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.

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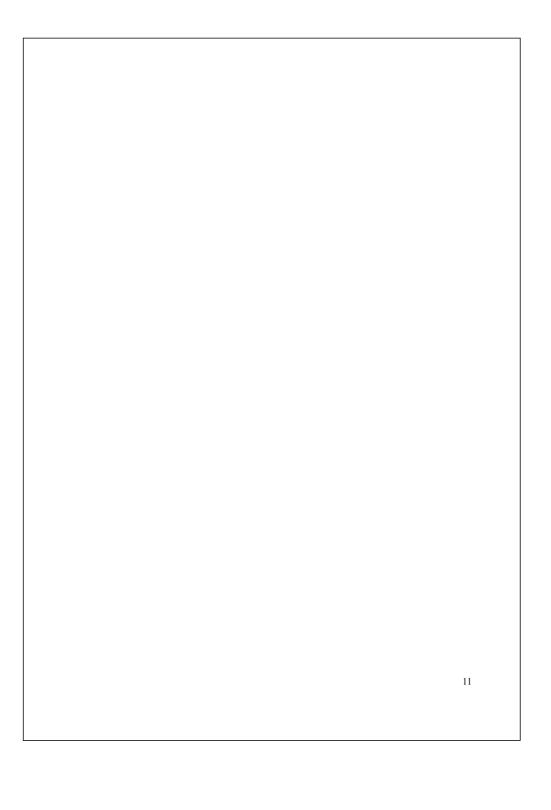
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