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EFFECTIVENESS OF SINGLE GARLIC EXTRACT ON FATTY LIVER AND LIVER ENZYMES IN RATTUS NORVEGICUS WISTAR

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Abstract

This study investigated the hepatoprotective efficacy of ethanol-extracted single garlic (EEBPT) on fatty liver and hepatic enzymes in rats. A true experimental design with pretest-posttest control group approach was employed. Twenty-four male Wistar rats (Rattus norvegicus) were randomly allocated into four groups: negative control (distilled water), positive control (simvastatin 2.1 mg/kg body weight), low-dose EEBPT (200 mg/kg body weight), and high-dose EEBPT (400 mg/kg body weight). All animals received high-fat diet supplementation for four weeks. Serum aspartate aminotransferase (SGOT/AST) and alanine aminotransferase (SGPT/ALT) were measured on days 0, 14, 21, and 28. Hepatic histopathology was assessed using hematoxylin and eosin staining. One-way ANOVA with Tukey post-hoc test and Kruskal-Wallis test were applied for statistical analysis ($p<0.05$). Results demonstrated that high-dose EEBPT significantly reduced SGOT and SGPT levels equivalent to simvastatin treatment and ameliorated hepatic steatosis, hepatocyte ballooning, and inflammatory infiltration. Ethanol-extracted single garlic at 400 mg/kg body weight exhibited hepatoprotective effects comparable to conventional statin therapy through antioxidative and anti-inflammatory mechanisms. Garlic extract represents a promising natural hepatoprotective agent for NAFLD management..

Keywords: *Allium sativum, Antioxidant, Fatty Liver, Garlic Extract, Hepatoprotective*

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INTRODUCTION

Phenomenon of Research

The escalation of body weight and adipose tissue accumulation represents a significant public health concern in contemporary society, primarily attributable to the widespread adoption of high-fat dietary patterns coupled with sedentary lifestyle behaviors. This metabolic shift generates excessive lipid accumulation both systemically and within hepatic parenchyma, substantially increasing the risk of metabolic dysregulation. Consequently, numerous metabolic complications emerge, including dyslipidemia, hypertension, and non-alcoholic fatty liver disease (NAFLD), collectively recognized as key manifestations of metabolic dysfunction. The pathophysiological cascade initiated by excessive nutrient intake creates a state of hepatic lipid overload, establishing conditions conducive to hepatocellular damage and progressive liver dysfunction. The prevalence of NAFLD has demonstrated exponential growth, paralleling the global epidemics of obesity and type 2 diabetes mellitus, positioning it as the most prevalent chronic liver disease worldwide, with substantial implications for public health management and clinical intervention strategies.

Non-alcoholic fatty liver disease is clinically characterized by macrovesicular lipid accumulation exceeding 5 percent to 10 percent of total hepatic weight in the absence of significant alcohol consumption. When inadequately managed, this condition exhibits potential for progressive advancement toward non-alcoholic steatohepatitis (NASH), a more severe phenotype characterized by hepatic inflammation, hepatocyte ballooning, and fibrotic remodeling, potentially culminating in cirrhosis and hepatic failure. During this pathological progression, oxidative injury to hepatocytes precipitates membrane integrity compromise, facilitating release of hepatic enzymes into systemic circulation. This phenomenon manifests as elevated serum concentrations of transaminases, including serum glutamic-oxaloacetic transaminase (SGOT/AST) and serum glutamic-pyruvic transaminase (SGPT/ALT), which serve as critical biomarkers for detecting hepatocellular injury and disease severity.

Problem Statement

The underlying pathogenic mechanisms driving NAFLD progression remain complex and incompletely understood, involving intricate interactions between genetic predisposition, environmental factors, and metabolic dysfunction. Current understanding incorporates the "two-hit hypothesis," wherein the initial insult comprises insulin resistance-mediated hepatic lipid accumulation, followed by secondary pathogenic mechanisms including oxidative stress, lipid peroxidation, and inflammatory cytokine production, collectively perpetuating hepatocellular damage. Contemporary evidence increasingly supports a "multiple-hit model," recognizing that NAFLD pathogenesis involves simultaneous dysregulation across multiple biological systems, including alterations in gut microbiota, compromised intestinal barrier integrity, hepatic autophagy dysfunction, and dysregulated hepatic stellate cell activation, all contributing synergistically to disease progression. The complexity of NAFLD etiology necessitates multifaceted therapeutic approaches extending beyond conventional pharmacological interventions, particularly given the current absence of disease-modifying pharmaceuticals with robust clinical efficacy and acceptable safety profiles.

Conventional synthetic therapeutic agents, such as statins and thiazolidinediones, have demonstrated limited efficacy in completely resolving NAFLD and frequently present significant adverse effects limiting long-term therapeutic utility. The inadequacy of current pharmacological strategies has catalyzed substantial investigative interest in botanical and naturally-derived compounds exhibiting hepatoprotective potential. Natural bioactive substances offer mechanistic advantages through polyvalent biological activities, including antioxidative, anti-inflammatory, and lipid-modulating properties, which collectively address multiple facets of NAFLD pathophysiology. Single garlic (Allium sativum L.), a widely cultivated bulbaceous plant with millennial utilization in traditional medicine systems, represents a particularly promising candidate for therapeutic investigation. This botanical source accumulates diverse organosulfur compounds and phytochemical constituents during tissue development, establishing a rich phytochemical profile supporting varied biological functions.

Single garlic demonstrates documented enrichment in bioactive organosulfur compounds including allicin, ajoene, and S-allylcysteine (SAC), alongside polyphenolic compounds such as flavonoids, which collectively exhibit potent antioxidative and anti-inflammatory pharmacological properties. The allicin compound, enzymatically generated through alliinase-mediated conversion of alliin upon cellular disruption, exhibits superior radical-scavenging capacity and demonstrates capacity to inhibit hepatic HMG-CoA reductase activity, thereby suppressing de novo cholesterol biosynthesis and reducing hepatic triglyceride accumulation. Complementarily, S-allylcysteine enhances endogenous antioxidative enzyme expression, including superoxide dismutase and glutathione peroxidase, while concurrently attenuating lipid peroxidation cascades triggered by hepatic lipid accumulation. These mechanistic pathways

suggest considerable potential for garlic extract to ameliorate NAFLD progression through multitargeted hepatoprotective effects.

Research Aim, Urgency, and Novelty

The present investigation aimed to systematically evaluate the hepatoprotective efficacy of ethanol-extracted single garlic (EEBPT) in a high-fat diet-induced fatty liver model utilizing Wistar rats (Rattus norvegicus), with particular focus on quantifying hepatocellular enzyme activity and histomorphological alterations. This research addresses a substantive knowledge gap regarding the dose-dependent hepatoprotective effects of garlic extract and its therapeutic equivalence relative to conventional synthetic hepatoprotective agents. The urgency of this investigation derives from the escalating global burden of NAFLD coupled with the absence of definitive pharmaceutical interventions and the urgent clinical imperative for identifying safe, efficacious, and economically accessible alternative therapeutic modalities. The novelty of this research resides in its rigorous comparative examination of garlic extract efficacy against simvastatin using both biochemical markers (transaminase quantification) and histopathological assessment, providing comprehensive evaluation of hepatoprotective mechanisms and clinical translational potential for natural product-derived therapeutics.

METHOD

1. Research Design and Method

This investigation employed a true experimental design with a pretest and posttest approach across multiple distinct groups, a methodological framework particularly suited for establishing causal relationships between independent variables and dependent outcome measures. True experimental designs constitute the most rigorous quantitative research approach, characterized by the deliberate manipulation of independent variables, random assignment of participants to experimental conditions, and the inclusion of both experimental and control groups, thereby providing superior capacity to establish internal validity and causal inferences compared to quasi-experimental or pre-experimental designs. The temporal structure of the investigation encompassed baseline assessment (pretest), treatment intervention administration, and subsequent outcome measurement (posttest), enabling systematic evaluation of treatment effects across four discrete temporal points. This methodological approach fundamentally differs from observational or correlational research designs by incorporating the essential elements of experimental control that strengthen the evidentiary basis for drawing conclusions regarding hepatoprotective mechanisms and comparative efficacy of garlic extract treatment protocols.

2. Study Population and Sample

This investigation utilized a total of twenty-four male Wistar rats (Rattus norvegicus), weighing approximately 200 grams each, with a mean age of approximately two months at study initiation. The selected animal model represents a widely validated biological system for investigating hepatic pathophysiology, metabolic dysregulation, and pharmacological interventions due to substantial physiological and genetic homology with human hepatic function and similar metabolic responses to dietary lipid load. The laboratory animal strain selection of Wistar rats demonstrates particular applicability for nutritional intervention studies because of their predictable growth patterns, reproducible metabolic responses, and well-characterized baseline physiological parameters that facilitate standardized experimental protocols and reliable outcome measurement.

The complete sample of twenty-four animals underwent randomization into four equitable experimental groups, each comprising six individual animals (n equals 6 per group). This sample size determination considered both statistical power requirements for detecting meaningful treatment effects and ethical obligations to minimize animal utilization in compliance with institutional animal research guidelines emphasizing the principle of animal welfare and minimization of animal numbers in research. Group assignments included the following: a negative control group (NC) receiving distilled water vehicle only; a positive control group (PC) receiving simvastatin at a dose of 2.1 milligrams per kilogram of body weight; a low-dose treatment group (P1) receiving ethanol-extracted single garlic at 200 milligrams per kilogram of body weight; and a high-dose treatment group (P2) receiving ethanol-extracted single garlic at 400 milligrams per kilogram of body weight. Random allocation to experimental groups was performed to eliminate selection bias and ensure comparability of baseline characteristics across treatment conditions, thereby strengthening the internal validity of experimental findings.

3. Research Instruments and Procedures

Garlic extract preparation constituted the initial procedural component and required standardized methodology to ensure consistency of bioactive compound composition across treatment administrations. Fresh bulbs of single garlic (Allium sativum L.) were subjected to ethanol extraction procedures utilizing established phytochemical isolation protocols optimized for recovery of organosulfur compounds and polyphenolic constituents. Dried garlic tissue was

ground into fine powder form to maximize surface area exposure during solvent contact. Approximately 100 grams of powdered garlic material was combined with 70 percent ethanol in a 1:10 weight to volume ratio, a solvent formulation specifically selected because binary ethanol water mixtures demonstrate superior extraction efficiency for diverse polyphenolic and organosulfur bioactives compared to either absolute ethanol or aqueous solvents alone. The preparation was subjected to maceration under controlled environmental conditions for 72 hours at ambient temperature with periodic agitation to facilitate solvent penetration and compound dissolution. Following maceration, the extract was filtered through Whatman filter paper, and the solvent was subsequently removed under reduced pressure using rotary evaporation to yield concentrated extract in semi-solid consistency. The dried extract was then dissolved in appropriate vehicle for precise dose calculation and administration to experimental animals.

All experimental animals were maintained under standardized laboratory conditions within the Integrated Pharmacology and Pharmacy Laboratory of Universitas Sumatera Utara from February through June 2025. Controlled environmental parameters included ambient temperature maintained between 20 and 26 degrees Celsius, relative humidity between 45 and 65 percent, and a standardized 12-hour light to dark photoperiod. All animals received standard laboratory pelleted diet supplemented with high-fat dietary components (butter and quail eggs) administered ad libitum to induce fatty liver pathology consistent with non-alcoholic fatty liver disease phenotype. Such dietary manipulation successfully generates hepatic lipid accumulation and associated metabolic dysfunction that parallels aspects of human NAFLD pathophysiology. Treatment interventions were administered via oral gavage at predetermined time intervals throughout the four-week experimental period. Blood samples were collected on predetermined assessment days (days 0, 14, 21, and 28) by cardiac puncture under general anesthesia maintained with intraperitoneal ketamine injection, ensuring humane animal handling and minimization of procedural discomfort in accordance with ethical guidelines for animal research.

Hepatic tissue sampling for histopathological examination was performed following final blood collection procedures on day 28. Liver specimens were immediately fixed in 10 percent neutral buffered formalin solution to preserve tissue histoarchitecture and prevent autolytic degradation. Fixed tissue underwent standard paraffin embedding procedures following conventional histological protocols. Tissue sections of 5 micrometer thickness were prepared using a rotary microtome and mounted on glass slides. Histopathological staining employed the hematoxylin and eosin (HE) technique, a foundational histological method enabling visualization and assessment of tissue structural elements, cellular morphology, and pathological alterations. The hematoxylin component provides basophilic staining of cellular nuclei and rough endoplasmic reticulum, while eosin contributes acidophilic staining of cytoplasmic proteins and extracellular matrix components, thereby generating comprehensive visualization of normal versus pathologically altered hepatic architecture including assessment of steatosis severity, hepatocyte ballooning phenomena, and inflammatory cell infiltration patterns.

4. Data Analysis and Statistical Methods

Serum samples obtained via cardiac puncture were centrifuged to separate cellular elements from liquid serum fraction. The serum was subsequently analyzed for hepatic enzyme activity using standardized laboratory methodology. Measurement of serum aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT) concentrations employed enzyme kinetic assays that quantify serum transaminase activity as a function of enzymatic catalysis of specific substrate reactions. These hepatic enzyme markers serve as critical surrogate biomarkers for assessing the magnitude of hepatocellular injury and integrity of hepatocyte membrane structures, as damage to hepatocytes precipitates release of these cytosolic enzymes into systemic circulation where they become detectable via serum analysis.

Quantitative data analysis proceeded through a structured protocol incorporating preliminary statistical assumption verification followed by hypothesis testing. Initial normality assessment of numerical outcome variables employed the Shapiro Wilk test, a powerful parametric normality assessment technique demonstrating superior discriminatory capacity compared to alternative normality testing procedures. Following confirmation of normal distribution of dependent variables (p greater than 0.05), analysis proceeded using parametric statistical methodology. Primary hypothesis testing employed one way analysis of variance (ANOVA), a parametric test procedure comparing mean values across three or more independent groups by partitioning total variance into between group and within group components. When ANOVA analysis revealed statistically significant differences among group means at significance threshold p less than 0.05, subsequent post hoc pairwise comparisons employed the Tukey honestly significant difference (HSD) procedure. The Tukey HSD test performs multiple pairwise comparisons while maintaining appropriate control over family wise type I error rates, thereby reducing the probability of falsely identifying statistically significant

differences when multiple comparisons occur, a methodological consideration of paramount importance in reducing false discovery rates within experimental analysis.

In instances where data distributions deviated from normality assumptions (p less than 0.05 on Shapiro Wilk testing), nonparametric statistical procedures substituted for parametric tests. The Kruskal Wallis test, a distribution free nonparametric alternative to one way ANOVA, compared medians across multiple independent groups without requiring assumptions of normality or homogeneity of variance, thereby providing robust statistical inference even when parametric test assumptions were violated. All statistical analyses employed IBM SPSS version 27.0 for Windows operating system software package (International Business Machines Corporation, Armonk, New York, USA). Statistical significance was established at conventional alpha error level of p less than 0.05, indicating that observed differences between groups had less than five percent probability of occurring by random chance alone.

Histopathological tissue samples were examined using light microscopy at standardized magnification, and qualitative assessment recorded the presence, severity, and spatial distribution of pathological findings including hepatic steatosis quantification (mild, moderate, or severe classification based on percentage of hepatocytes containing lipid vacuoles), hepatocyte ballooning phenomena characterized by cellular enlargement with pale cytoplasm, and mononuclear inflammatory cell infiltration patterns. Histopathological findings were systematically compared across experimental groups to establish temporal progression of liver damage in control animals and treatment related reversal of pathological changes.

RESULTS AND DISCUSSION

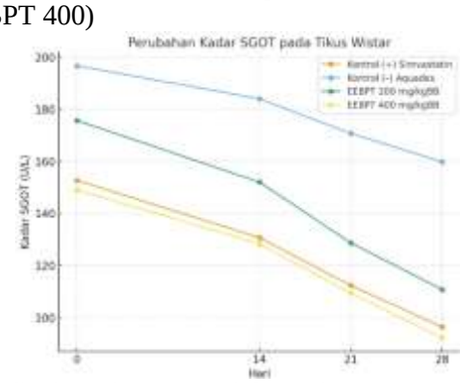
Sebanyak 24 ekor tikus jantan ekor putih (Rattus norvegicus Wistar) yang digunakan Dalam penelitian ini, hewan uji memiliki berat badan sekitar 200 gram dengan rata-rata usia kurang lebih 2 bulan. Masing-masing dari keempat kelompok tikus menerima enam ekor yang berbeda. Selama penelitian berlangsung tidak ditemukan adanya hewan uji yang drop out atau mati, sehingga semua sampel dapat digunakan sampai akhir penelitian.

Aktivitas enzim hati (SGOT dan SGPT) berdasarkan hasil pemeriksaan

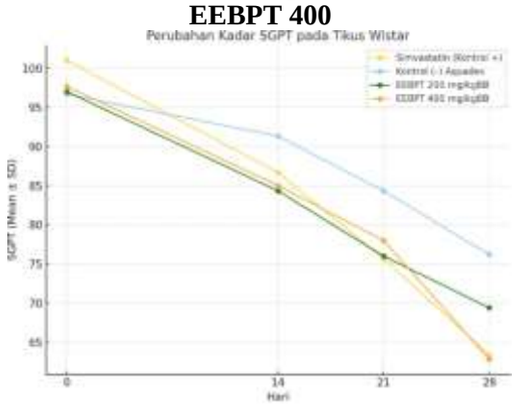
Kami menguji serum tikus Wistar untuk kadar enzim hati (SGOT dan SGPT) pada hari ke-0, 14, 21, dan 28.

Tabel 1. (Mean ± SD) kadar rata-rata enzim SGOT dan SGPT serum tikus Wistar pada hari ke-0, 14, 21, dan 28.

Grafik 1 garis khusus SGOT tiap kelompok → Simvastatin, Kontrol, Aquades, EEBPT 200, EEBPT 400)



Grafik 2 garis khusus SGPT tiap kelompok → Simvastatin, Kontrol, Aquades, EEBPT 200, EEBPT 400



Interpretasi:

Grafik 1 menunjukkan bahwa kadar SGOT mengalami penurunan bertahap pada kelompok Simvastatin dan EEBPT, sedangkan kelompok kontrol cenderung meningkat**. Grafik 2** memperlihatkan pola serupa pada SGPT, di mana perlakuan EEBPT dosis 400 mg/kgBB memberikan efek penurunan yang mendekati Simvastatin."

Tabel 2. Hasil Uji One Way ANOVA Kadar SGOT dan SGPT Serum Tikus Wistar

Parameter	Hari ke-0		Hari ke-14		Hari ke-21		Hari ke-28	
	F	p	F	p	F	p	F	p
SGOT	1.882	0.211	2.783	0.110	3.923	0.054	14.927	0.000
SGPT	0.105	0.955	0.409	0.751	0.447	0.726	0.405	0.018
Keterangan	F (F-ratio): nilai statistik hasil uji ANOVA yang menunjukkan besarnya variasi antar kelompok dibandingkan dengan dalam kelompok.							
	p(signifikansi): probabilitas hasil perbedaan terjadi secara kebetulan.							

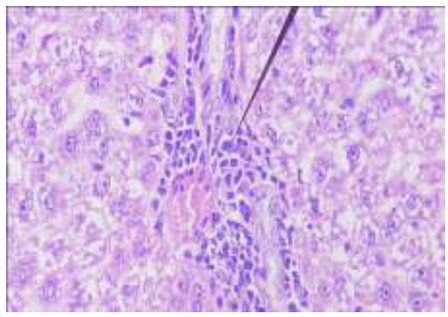
Pada hari ke-28, tampak perbedaan yang signifikan ($p < 0,05$) di antara seluruh kelompok, sebagaimana ditunjukkan melalui hasil uji statistik (ANOVA).

Tabel 3. Kadar SGOT dan SGPT serum tikus Wistar pada hari ke-28 berdasarkan hasil Uji Post Hoc Tukey

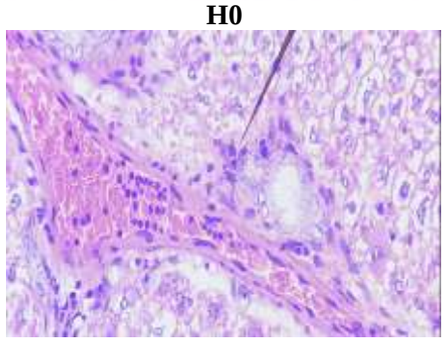
Kelompok Dbandingkan	Dosis (mg/kgBB)	Parameter	Mean ± SD	p value	Keterangan
Aquades vs Simvastatin	0,09	SGOT	63.40	0.001*	Bermakna
		SGPT	14.20	0.031*	Bermakna
Aquades vs EEBPT	200	SGOT	49.00	0.003*	Bermakna
		SGPT	8.20	0.042*	Bermakna
	400	SGOT	67.60	0.000*	Bermakna
		SGPT	14.80	0.025*	Bermakna
Simvastatin vs EEBPT	200	SGOT	-14.40	0.521	Tidak bermakna
		SGPT	-6.00	0.378	Tidak bermakna
	400	SGOT	4.20	0.887	Tidak bermakna
		SGPT	0.60	0.922	Tidak bermakna

Dari hasil uji Post Hoc Tukey pada hari ke-28, diperoleh bahwa: Dibandingkan dengan kelompok kontrol negatif yang memperoleh aquades, seluruh kelompok perlakuan menampilkan perbedaan bermakna secara statistik ($p < 0,05$). yaitu Simvastatin, Terjadi perbedaan signifikan pada SGOT dan SGPT antara kontrol negatif dan kelompok EEBPT dosis 200 mg/kgBB serta 400 mg/kgBB. Di sisi lain, Simvastatin, EEBPT 200 mg/kgBB, serta EEBPT 400 mg/kgBB tidak menunjukkan perbedaan signifikan satu sama lain ($p > 0,05$). Hal ini mengindikasikan bahwa EEBPT pada dosis 200 mg/kgBB atau 400 mg/kgBB memberikan efek pelindung yang setara dengan Simvastatin dalam menekan kadar enzim hati.

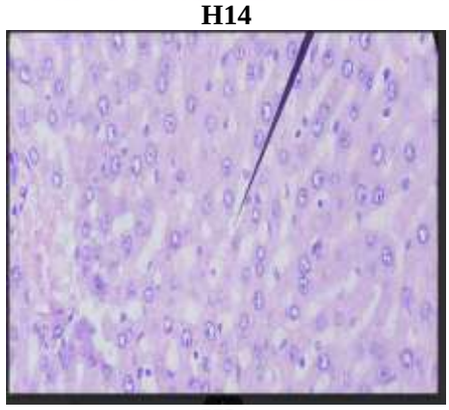
Tabel 4. Gambaran Histopatologi Hati Tikus Wistar



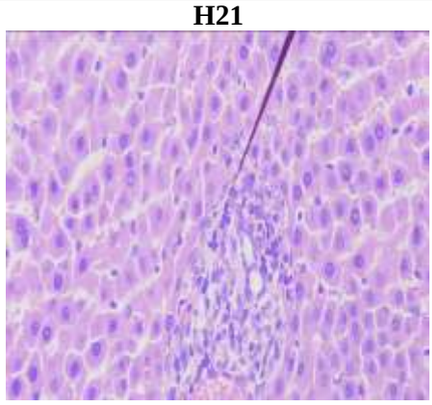
H0



H14



H21



H28

Keterangan: H0: Pemberian ekstrak bawang putih tunggal terhadap fatty liver dan ezim hati pada hari ke-0; H14: Pemberian ekstrak bawang putih tunggal terhadap fatty liver dan ezim hati pada hari ke-14; H21: Pemberian ekstrak bawang putih tunggal terhadap fatty liver dan ezim hati pada hari ke-21; H28: Pemberian ekstrak bawang

putih tunggal terhadap fatty liver dan ezim hati pada hari ke-28

Selain pemeriksaan biokimia, gambaran histopatologi hati memberikan bukti visual mengenai tingkat kerusakan hepatosit. Pada awal penelitian (hari ke-0), hampir seluruh sampel menunjukkan kerusakan hati berupa steatosis sedang–berat, ballooning hepatosit, dan infiltrasi sel radang mononuklear. Kondisi ini konsisten dengan perubahan histopatologi pada fatty liver disease.

Setelah 28 hari perlakuan, kelompok kontrol negatif tetap menunjukkan kerusakan hati yang jelas. Sebaliknya, pada kelompok perlakuan dengan EEBPT dosis 200 mg/kgBB tampak adanya perbaikan dengan steatosis yang lebih ringan dan berkurangnya infiltrasi sel radang. Kelompok dosis 400 mg/kgBB menunjukkan perbaikan paling optimal: steatosis minimal, ballooning jarang ditemukan, dan peradangan berkurang hanya menjadi 1–2 fokus. Gambaran ini hampir menyerupai kelompok simvastatin.

CONCLUSION

This investigation systematically demonstrated that ethanol-extracted single garlic (EEBPT) possessed substantial hepatoprotective efficacy in a high-fat diet-induced fatty liver model, with dose-dependent therapeutic effects comparable to simvastatin treatment. Primary findings revealed that EEBPT administration at both 200 milligrams per kilogram body weight and 400 milligrams per kilogram body weight significantly reduced hepatic enzyme markers, specifically serum aspartate aminotransferase and alanine aminotransferase, with maximal therapeutic benefit observed at the 400 milligrams per kilogram dose, exhibiting biomarker suppression equivalent to simvastatin pharmacotherapy. Histopathological examination corroborated biochemical findings, demonstrating that high-dose garlic extract administration substantially ameliorated hepatic steatosis severity, reduced hepatocyte ballooning phenomena, and minimized inflammatory infiltration patterns compared to untreated controls. These results provide compelling preliminary evidence that naturally-derived bioactive compounds, particularly organosulfur constituents including allicin and S-allylcysteine, exert multitargeted hepatoprotective mechanisms through synergistic antioxidative and anti-inflammatory pathways, effectively halting progression from simple hepatic steatosis toward non-alcoholic steatohepatitis phenotypes. The therapeutic equivalence between garlic extract and synthetic statin therapy suggests potential clinical utility as an alternative hepatoprotective intervention strategy.

However, several important methodological limitations warrant acknowledgment when interpreting these findings. This research employed an animal model utilizing Wistar rats, which, although demonstrating physiological homology with human hepatic function, exhibits species-specific metabolic variations potentially limiting direct clinical extrapolation to human populations. The relatively short four-week experimental duration does not address long-term hepatoprotective sustainability or cumulative bioavailability patterns observed in extended clinical applications. Future investigations should prioritize randomized controlled trials in human populations with established non-alcoholic fatty liver disease, incorporate standardized garlic extract formulations with rigorously characterized organosulfur composition, and evaluate dose-dependent responses across extended intervention periods. Additionally, mechanistic studies employing advanced molecular techniques should elucidate specific signaling pathways mediating garlic extract hepatoprotection, potentially identifying biomarkers predicting individual therapeutic responsiveness. Such investigations would facilitate evidence-based clinical translation of natural product therapeutics and establish garlic extract as a safe, economically accessible, and efficacious dietary supplement for NAFLD

management and prevention within diverse population cohorts.

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