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The Effect of Quercetin on Coenzyme HMG-CoAR, ABCA1 Transporter, Dyslipidemia Profile and Hepatic Function in Rats Dyslipidemia Model

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Abstract

Background*: Dyslipidemia is a lipid metabolic disorder that increases the risk of cardiovascular disease, typically marked by abnormalities in triglycerides (TG), low-density lipoprotein (LDL), and total cholesterol (TC), along with decreased high-density lipoprotein (HDL) levels. This study explored the potential of quercetin, a natural substance, as a preventive agent against dyslipidemia induced by high-fat diet (HFD) in a rat model. Simvastatin, a standard cholesterol-lowering drug, was used for the comparison.* ***Objective:*** *The main objective of this research was to evaluate the potential of quercetin in lipid metabolism for dyslipidemia caused by HFD and compare its effects with the first-line drug therapy simvastatin, which has a similar mechanism.* ***Methods:*** *Rats fed a HFD were treated with quercetin and simvastatin, and their lipid profiles, liver enzyme activities, and molecular markers related to cholesterol metabolism were analyzed.* ***Results:*** *Quercetin markedly decreased cholesterol levels by inhibiting the enzyme 3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMG CoAR). Cellular observation revealed that it also prevented liver damage and showed a protective effect on liver enzyme activity. Quercetin enhanced the expression of the Adenosine Triphosphate Binding Cassette subfamily A member 1 (ABCA1) protein, showing a protective effect against dyslipidemia akin to simvastatin, yet with a reduced likelihood of liver toxicity.* ***Conclusion:*** *Quercetin may serve as an effective and safer alternative to simvastatin for treating dyslipidemia, offering cholesterol-lowering benefits without hepatotoxic risks associated with long-term statin therapy.*

Keywords*: ABCA1, cholesterol, HMG-CoAR, quercetin, simvastatin*

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INTRODUCTION

Dyslipidemia, defined by elevated levels of TC, LDL, or TG, often alongside reduced HDL cholesterol levels, is commonly assessed using these lipid parameters to evaluate cardiovascular risk (Hedayatnia et al., 2020). Cardiovascular disease is a leading cause of mortality worldwide, with abnormalities in cholesterol levels constituting a significant factor (Perki, 2022). Worldwide, cardiovascular diseases are responsible for approximately 3.9 million fatalities each year (NCD, 2020). A mortality study by Yi et al. (2018) examined the relationship between total cholesterol levels and overall mortality in a cohort of 12.8 million people in Korea. The data indicate that elevated TC levels are associated with a heightened risk of mortality (Yi et al., 2019). Similarly, epidemiological studies in the U.S. have demonstrated a correlation between cholesterol levels and the mortality risk from heart attack and stroke (Jeong et al., 2018). Moreover, data from the American Heart Association (AHA) indicated that cholesterol testing decreased by over 39% in 2020 (Barnard et al., 2019), highlighting concerns about monitoring and managing cholesterol levels.

The process of cholesterol entry into the bloodstream is complex, with a key mechanism involving digestion of dietary fats facilitated by chylomicrons. Cholesterol is produced by different cells in the body and is essential for various cellular functions. The liver is central to cholesterol biosynthesis and is the main site of de novo cholesterol production. This process is crucial for maintaining cholesterol homeostasis, reflecting the essential role of the liver in regulating the body's cholesterol supply (Huff et al., 2023). Cholesterol production begins with the generation of acetyl-CoA from two Ac-CoA molecules. HMG-CoA synthase transforms this molecule into HMG-CoA, which is subsequently reduced to mevalonate. Mevalonate is then subjected to phosphorylation and decarboxylation to form IPP, which further polymerizes to form FPP. This process diverges into three pathways: (1) two FPP molecules condense to form squalene, a precursor to cholesterol; (2) IPP combines with FPP to form polyprenyl derivatives for ubiquinone, and (3) additional IPP units combine with FPP to produce dolichol. Cholesterol is synthesized in the endoplasmic reticulum (Shi et al., 2022). LDL transports cholesterol through the bloodstream. LDL carries cholesteryl esters (CEs) that bind to LDL receptors (LDLRs) on the cell membrane. After absorption, CEs are hydrolyzed in lysosomes and free cholesterol is transported to various organelles, including the endoplasmic reticulum and mitochondria (Shi et al., 2022). Cholesterol accumulation can lead to mitochondrial dysfunction, apoptosis, and tissue necrosis, contributing to the formation of atherosclerotic plaques, which is a hallmark of cardiovascular disease (Hill et al., 2023). Key transporters such as ABCA1 and ABCG1 regulate HDL metabolism. The expression of ABCA1 is modulated by compounds, such as cAMP and nuclear receptors. Augmented expression facilitates reverse cholesterol transfer, elevates HDL levels, and diminishes atherosclerotic plaques (Wang et al., 2019; Zhang et al., 2016).

The primary treatment for dyslipidemia often involves statins (Perki, 2022). Similar to other statins, simvastatin works by inhibiting the enzyme HMG-CoAR, which is crucial for producing mevalonate, a key precursor in cholesterol biosynthesis (Parihar et al., 2019). Statins inhibit cholesterol synthesis and isoprenoid formation by interfering with the mevalonate pathway (Parihar et al., 2019). However, statin therapy has side effects, including the risk of rhabdomyolysis, a condition caused by mitochondrial dysfunction in muscle cells, leading to apoptosis (Bouitbir et al., 2020; Mollazadeh et al., 2021). Additionally, liver toxicity is a concern in statin use as it can exacerbate cirrhosis and may result in proteinuria (Ward et al., 2019). Quercetin, a flavonoid, is emerging as a promising therapeutic agent for the management of cholesterol levels. Its function in cholesterol metabolism involves inhibiting HMG-CoAR activity, reducing SREBP-1c function, and enhancing the actions of transporter proteins ABCA1 and ABCG1. Quercetin also shows potential in improving ischemic stroke outcomes by upregulating MC4R and scavenging free radicals. Research has underscored the efficacy of quercetin as a therapeutic agent for dyslipidemia (Zhang et al., 2016; Chamber et al., 2019; Rahmadi et al., 2020; Perki, 2022). Quercetin is a potential candidate for modulating lipid metabolism as it can influence downstream pathways by targeting both HMG-CoAR and ABCA1. Quercetin reduces lipid storage by downregulating HMG-CoAR through the upregulation of the AMPK pathway, which decreases cholesterol biosynthesis and promotes cellular energy balance. Additionally, it enhanced lipid oxidation by upregulating the PPAR pathway, which governs fatty acid metabolism and mitochondrial function. In the long term, these mechanisms contribute to improved lipid homeostasis and a reduced risk of atherogenic processes, demonstrating the interplay between cholesterol biosynthesis, lipid oxidation, and energy regulation. (Chamber et al. 2019; Wang et al. 2021).

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The 2016 study by Zhang et al. had limitations, particularly in validating quercetin levels in the blood, because the compound was mixed with rat feed, leading to inconsistent intake. Additionally, blood quercetin levels were not measured, rendering cholesterol-related conclusions unclear. This new study will involve the oral administration of quercetin for accurate dosing. This research will focus on the effects of quercetin on suppressing HMG-CoAR expression and increasing ABCA1 expression, while observing lipid profiles (TC and TG) and liver function (cellular observation, aspartate aminotransferase (AST), and alanine aminotransferase (ALT)). This study aimed to improve previous findings and to assess the potential of quercetin as an anti-cholesterol treatment.

MATERIALS AND METHODS

Materials

Simvastatin (PT. Dexa Medica, Indonesia), and quercetin (Sigma-Aldrich, Singapore) were solubilized in Aquadest (Interchemie, Netherlands) and CMC-Na (PT. Jong Java Chemicals Ltd, Indonesia), fat powder that consisting of 98% palm oil fat and beta carotene 2 % (Rojokoyo group, Indonesia). Quercetin at doses of 200, 100, and 50 mg/kg BW, together with simvastatin at 20 mg/kg BW, were orally administered for 36 days. The normal chow group received standard rat food, whereas the HFD group was provided with rat food supplemented with 70% fat powder to induce dyslipidemia (Udomkasemsab et al., 2018). Figure 1 shows the timeframes and protocols.

Methods

Animals

This study utilized male Wistar rats weighing 190–210 g aged between 150 and 300 days (Castillo et al., 2018; Ghasemi et al., 2021). The sampling method involved randomly allocating rats into six treatment groups. Rats were maintained in cages at 19-22 °C, with 50-70% humidity levels, and subjected to a 12-hour dark-light cycle (Zhang et al., 2016; Li et al., 2023).

The research method to be carried out was a modification of the research conducted by Zhang et al. *in* 2016. The sample sizes were calculated based on the Ferderer rule to produce significant data with the addition of error anticipation. Forty-two rats were randomly allocated into six groups: normal chow, HFD, quercetin at three doses, and simvastatin. Normal chow does not receive treatment and will be given a normal diet without added fat, compared to the healthy placebo group. The HFD, quercetin, and simvastatin groups were fed a diet containing added fat. Every group will undergo treatment for 5 weeks to develop dyslipidemia, and body weight and rat feed will be measured each day (Udomkasemsab et al., 2018). The dosages of quercetin and simvastatin were determined according to the effective doses established in prior research aimed at ameliorating dyslipidemia (Zhang et al., 2016; Zhang et al., 2020; Papakyriakopoulou et al., 2022). The quercetin cohort will be stratified into three dosage groups: 200 mg/kg BW, 100 mg/kg BW, and 50 mg/kg BW, from the lowest dosage that affects the dyslipidemia profile to the highest dosage (Papakyriakopoulou et al., 2016; Papakyriakopoulou et al., 2022). Simvastatin was administered at 20 mg/kg BW, which reduced cholesterol levels during HFD induction (Zhang et al., 2020). The cohort administered quercetin and simvastatin will receive the medication orally daily, according to the specified dosage for each group, and will be compared with the HFD group that did not receive the medication. After 36 days, each group was anesthetized with ketamine-xylazine compounds at 100 and 10 mg/kg doses via intraperitoneal administration (Castillo et al., 2018; Linsenmeier et al., 2020).

The rats were then dissected to collect blood and isolate the liver for polymerase chain reaction (PCR) (Susanti et al., 2019; Lei et al., 2020). Blood was stored in a tube before centrifugation, and then the serum was collected and stored at a storage temperature of 4 °C. The examination must occur within 24 h (Layssol-lamour et al., 2019). The liver was preserved in a 0.5 ml tube with liquid nitrogen and maintained at cold stored at -80 °C (Oertel et al., 2006; Lei et al., 2020). All experimental procedures were approved by the Faculty of Veterinary Medicine Ethics Committee at Universitas Airlangga (ethical number 2) KEH.001.07.2024.

Identification of dyslipidemia profile

The procedure was conducted by centrifuging the sample for 10 min to isolate serum from the plasma. The serum from the preserved tube was then subjected to photometric analysis. The kit was prepared at 37°C following the standard solution according to the cholesterol profile parameters to be observed. Next, pipette ten µL of the serum solution was pipetted into a sample tube. Once the tube cap was opened, it was placed in the vacuum section of the sample. The vacuum draws the sample in, allowing for observation of each cholesterol profile level. The measured profiles included total cholesterol and triglyceride levels.

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Identification of aspartate aminotransferase and alanine transaminase profiles

The working reagent was prepared by pipetting according to the profile parameters and the sample into a 1.5 mL tube, ensuring that the mixture was protected from light. The working reagent and sample were placed into a heat blocker at 37°C for 5 min. The working reagent was then added to each sample, homogenized, and centrifuged. Next, the working reagent and the working reagent-sample mixture were pipetted into a microplate and protected from light. The microplate was incubated at 37°C for 1 min. Subsequently, a microplate reader was used to measure absorbance at wavelengths below 340 nm. The reaction was allowed to progress for one minute, and the absorbance was measured four times in total, comprising one initial reading and three subsequent readings.

Liver cellular observation

Formalin-fixed tissue samples were prepared for hematoxylin and eosin staining by rinsing in distilled water to remove residual tissue, followed by rehydration with alcohol and immersion in water. Hematoxylin staining was performed by immersing the tissue in hematoxylin solution for 5 – 10 min, with excess stain removed via a tap water rinse and optional differentiation using acid alcohol to eliminate nonspecific staining. The tissue was treated with bluing solution to enhance nuclear staining, followed by rinsing with water. Eosin staining was conducted by immersing the slides in Eosin Y solution for 1–2 min, with staining intensity adjusted using a brief rinse in distilled water or diluted alcohol. Dehydration was performed using alcohol followed by clearing with xylene or a substitute. Finally, the slides were mounted with a compatible mounting medium, and coverslips were applied to complete the preparation for microscopic examination. Observations were then conducted under a microscope at 200x and 400x magnifications to assess steatosis and inflammation. Steatosis is marked by lipotoxicity, characterized by clear, round vacuoles within the cytoplasm of hepatocytes, and inflammation. Inflammation is marked by hyperplasia of Kupffer cells and neutrophil infiltration.

Quantitative polymerase chain reaction (qPCR)

Animals were euthanized using ketamine and xylazine 36 days after the initiation of the HFD. Blood and liver tissues were collected and preserved in liquid nitrogen (LN). The samples were stored at −80°C. RNA was then extracted from the sample using a Total RNA Purification Kit (Jena Bioscience, Germany) and a Quantus Fluorometer (Promega, U.S.) was used to make sure RNA was distributed equally. The RNA reverse transcription method was used with a GoScript™ Reverse Transcriptase Kit (Promega) to generate cDNA. QRT-PCR was used to evaluate HMG-CoAR mRNA expression. Antisense primer: 5′-AGAGCTGGCTTGAAACACCTGA-3′; sense primer: 5′-TTTGGA CTGGAGACGGATGTAGTAGA-3′ for the target gene, and for ABCA1, antisense primer: 5′-CCAGGAGCGTGTGAGCAAAG-3′; sense primer: 5′-ACCAGTGTAGCAGGGACCACATAA-3.' The β-actin gene, antisense primer: 5′-TTCTTGGGTATGGAATCCTGT -3′; sense primer: 5′-AGCACTGTGTTGGCATAGAG -3′ functioned to normalize HMG-CoAR and ABCA1 mRNA expression. GoTaq RT qPCR Master Mix Kit (Promega) was used for qRT-PCR, and gene expression alterations were assessed using the 2-ΔΔCt method to determine cycle threshold (Ct) values.

Statistical analysis

Data analysis was performed using GraphPad Prism software. A one-way ANOVA was conducted to evaluate triglycerides, total cholesterol, aspartate transferase, alanine transferase, and the relative expression levels of HMG-CoAR and ABCA1 mRNA if there was any statistically significant. Dunnett post hoc test was used to determine which specific groups differed from each other. Hepatotoxic markers, such as aspartate transferase, alanine transferase, and cellular observation sample size candidates were carried out based on potential rat gene expression analysis. Two-way ANOVA was used to collect data on body weight and feed consumption. If there was any statistically significant difference, Dunnett's post-hoc test was used to determine which groups differed from each other. The significance value was first calculated and then compared against the 95% confidence level to assess the statistical hypotheses ($P < 0.05$). Animal group allocation in research designs that incorporate randomization, replication, and the existence of treatment and control groups follows a structured approach to ensure the study's validity and reliability.

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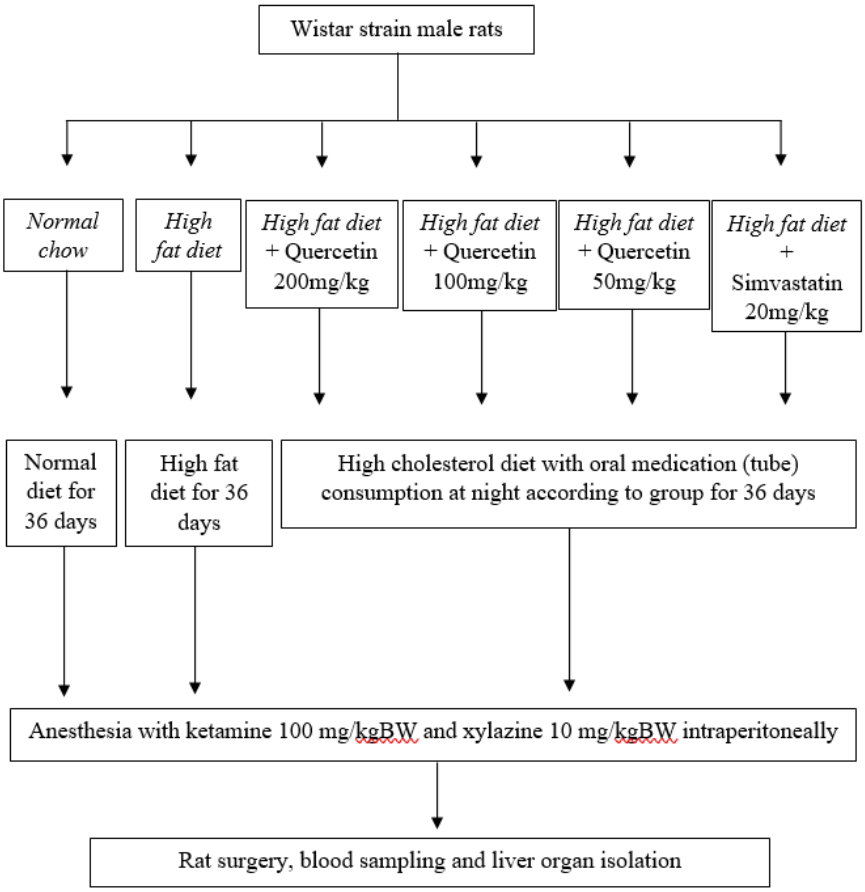

Figure 1. The timetable and dosing regimen for the trial were delineated as follows. Following the assessment of body weight and feed intake of the rats on day -1, the subjects were administered an HFD to induce dyslipidemia. The treatment group was orally administered quercetin at three different dosages: 200 mg/kg BW, 100 mg/kg BW, and 50 mg/kg BW, in conjunction with simvastatin at 20 mg/kg BW. The rats were killed on day 36, and blood samples, along with liver tissues, were obtained

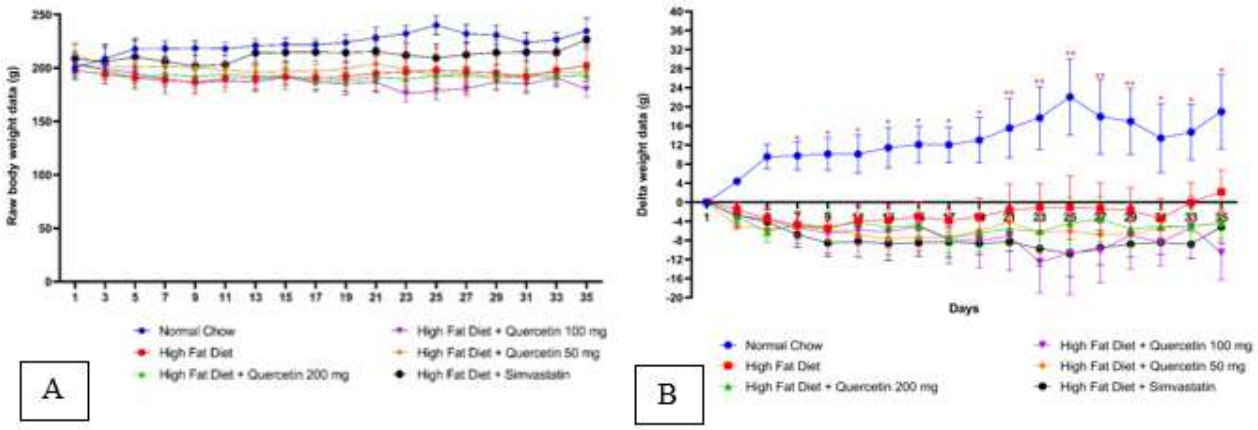

Figure 2. Comparison of body weight profiles among HFD, treatment, and normal chow groups. Each point represents the average body weight of each group (Figure 2A), and the change in delta weight (Figure 2B) is presented as the mean ± SEM. **p<0.01 and *p<0.05 in comparison to HFD. Two-way ANOVA and Dunnett post-hoc analysis; n = 3 – 7 rats

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RESULTS AND DISCUSSION

The effect of giving HFD on the body weight of rats

Changes in body weight due to the implementation of an HFD illustrate the body's response to HFD consumption. In this study, the HFD was administered for 36 days, following previous experiments, to trigger dyslipidemia marked by irregular cholesterol levels in the bloodstream. The rats' body weight measurements are presented in Figure 2 A, with weighments performed over 36 days. These observations indicated that the normal chow group had a consistent weight gain relative to the HFD group, with disparities evident as early as day 3. Figure 2 B illustrates a marked change in the body weight delta on day 7 in the HFD group, with a drop of +8 grams relative to the normal chow group. During the subsequent days, the group exclusively receiving HFD exhibited a notable reduction in weight during the 15-day observation period compared with the normal chow group ($p < 0.05$, $p < 0.01$), ultimately reverting to their baseline body weight. No significant changes were found in the HFD + quercetin 200 mg and HFD + quercetin 50 mg groups compared to the HFD group over 36 days, and the rats' body weight returned to initial levels. Nevertheless, the HFD + quercetin 100 mg group did not demonstrate significant alterations over 36 days compared to the HFD group, and their body weight did not revert to the baseline level, resulting in a total decrease of $\pm$12 grams. Likewise, the HFD + simvastatin cohort exhibited no significant alterations relative to the HFD group and reverted to baseline weight throughout the 36-day observation period (Figure 2).

This study aimed to determine the effect of quercetin on dyslipidemia, changes in molecular profiles, and hepatic function in a rat model of HFD. This study sought to determine the efficacy of quercetin and simvastatin as prophylactic interventions for dyslipidemia. The study began by observing the rats' body weight and feed consumption, comparing these parameters to every treatment group and the HFD group. The results showed that all groups, including the untreated HFD group, returned to baseline body weight over time. The main goal of administering quercetin is to evaluate its ability to prevent metabolic disorders, typically signalled by shifts in body weight. This study was performed to determine the effect of quercetin on dyslipidemia, changes in molecular profiles, and hepatic function in a rat model of HFD. This study aimed to assess the efficacy of quercetin and simvastatin as prophylactic interventions for dyslipidemia. This weight loss may be attributed to ketosis, which can occur in rats fed a diet with a fat content of 65-75% (Bielohuby et al., 2011; Modica et al., 2021).

Subsequent studies evaluated the effects of three distinct dosages of quercetin and simvastatin on the HFD group. Despite the absence of notable variations in body weight across the quercetin, simvastatin, and HFD groups, quercetin demonstrated the capacity affected fat metabolism with a minimal risk of rhabdomyolysis. The protective effect of quercetin is probably due to its ability to decrease cholesterol buildup and stop ATP depletion, which aids in maintaining standard levels of critical metabolic regulators, such as PGC-1α, UCP2, and PPARγ (Mollazadeh et al., 2021; Papakyriakopoulou et al., 2022; Yi et al., 2021).

The effect of giving HFD on rats feed consumption

Daily feed consumption of the experimental animals was tracked to assess their food intake patterns. This tracking was performed to ensure that variations in feed composition did not affect the eating patterns of the rats. Daily measurements were obtained by determining the change in feed weight from one day to the next, starting on day 2. The results of these measurements over 36 days are shown in Figure 3. The HFD group showed no significant difference in food consumption compared with the usual chow group. A subsequent study indicated that the HFD-only group sustained consistent feed intake rates of 90-100%. The HFD + quercetin 200 mg group exhibited a significant increase in feed consumption over three days ($p < 0.05$), with intake escalating to 120-140%. The HFD + quercetin 100 mg group exhibited no significant alterations over the 36-day observation period, with consumption levels ranging from 100% to 120%. The HFD + quercetin 50 mg group demonstrated significant increases in feed consumption over 10 days, ranging from 120% to 140%, with $p < 0.01$ and $p < 0.05$, respectively. Ultimately, the HFD + simvastatin cohort exhibited no significant alterations in food consumption over the 36-day observation period, maintaining intake levels between 100-120% (Figure 3).

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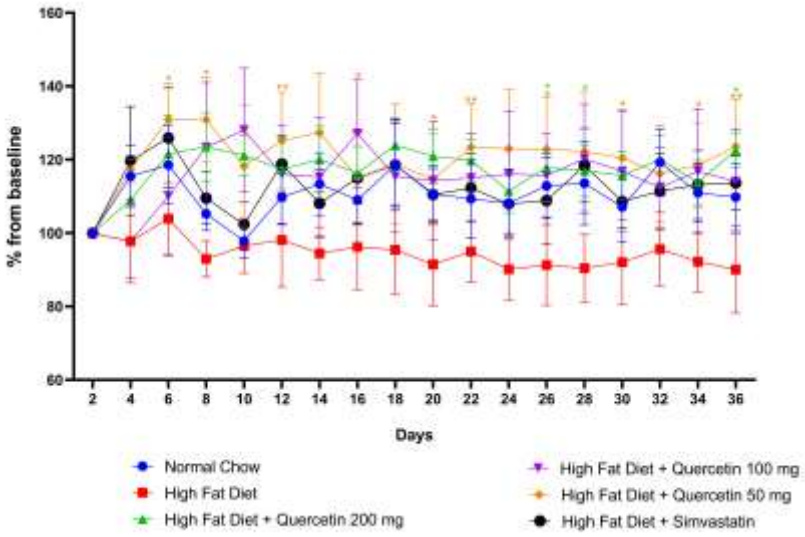

Figure 3. Comparison of feed weight profiles among HFD, treatment, and normal chow groups. Each point represents the average feed weight of each group and is presented as the mean ± SEM. **p<0.01 and *p<0.05 in comparison to HFD. Two-way ANOVA and Dunnett post-hoc analysis; n = 3 – 7 rats

Table 1. Table of the effect of quercetin and simvastatin on triglyceride levels in rat plasma after induction of HFD feeding.

Group	Triglyceride level (mg/dL)
Normal chow	21,14 ± 5,27
HFD	21,83 ± 6,03
HFD + Quercetin 50 mg	26,80 ± 6,18
HFD + Quercetin 100 mg	37,67 ± 15,44
HFD + Quercetin 200 mg	30,00 ± 9,03
HFD + Simvastatin	23,00 ± 15,39

***K :** The data presented are the mean ± SEM of 3 - 7 rats every group

Abnormalities in lipid metabolism characterize dyslipidemia and can be influenced by factors such as genetics, the environment, lifestyle, and diet (Pappan et al., 2024). In this study, the feeding habits of the HFD group and those on normal chow exhibited no notable differences, suggesting that the recorded weight loss was not attributed to variations in the rats' appetite. HFD stimulates gastric leptin, leading to a feeling of fullness and increased calorie burning (Mendoza et al., 2021). Even with a similar food intake, calorie balance can influence weight loss (Hall et al., 2018). Further analysis revealed a significant difference between the HFD + quercetin 200 mg group over 3 days and the HFD + quercetin 50 mg group over 10 days compared with the HFD group ($p < 0.01$ and $p < 0.05$). These disparities may be ascribed to the capacity of quercetin to suppress leptin, which modulates appetite via the leptin signalling system (Klok et al., 2007; Wang et al., 2024). Although simvastatin did not affect the rats' appetite, it may have altered the gut microflora in their stomachs (Zhang et al., 2020).

Quercetin administration effect on TG levels in plasma of rats induced by HFD

TG profiles were analyzed to evaluate the impact of HFD induction on plasma TG levels. This study aimed to determine the preventive efficacy of quercetin in reducing the alterations in triglyceride levels after HFD induction. The data presented in Table 1 demonstrate that HFD did not substantially alter plasma TG levels in rats. A subsequent study contrasted the HFD group with HFD groups administered quercetin to assess the prophylactic effect of quercetin on hypertriglyceridemia. The results showed no significant increase in TG levels in the HFD group relative to that in the normal chow group, suggesting that hypertriglyceridemia was not adequately induced. In the HFD + quercetin groups (50, 100, and 200 mg), TG levels increased, although no significant difference was found when compared to the HFD group. Similarly, the

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HFD + simvastatin cohort exhibited elevated TG levels. However, there was no statistically significant difference compared with the HFD group (Table 1).

Dyslipidemia is a medical condition marked by elevated TG and TC levels. These parameters are typically measured to assess an individual's risk for cardiovascular disease. An anomaly in lipid levels elevates cardiovascular disease risk factors and is a leading cause of global mortality (Hedayatnia et al., 2020). Dyslipidemia is a risk factor for several heart diseases (Perki, 2022). In this study, TG profile observations were conducted to determine the effect of HFD on lipid levels and the potential preventive effects of quercetin and simvastatin were evaluated (Yuan et al., 2020; Papakyriakopoulou et al., 2022). A comparison between the normal chow and HFD groups revealed no significant variations in triglyceride levels. Previous research has indicated an elevation in triglyceride levels resulting from HFD induction. However, the lack of a significant difference in this study may be attributed to the type of fat used in the feed, which may not have stimulated fatty acid-binding protein 2, thereby preventing TG resynthesis and peripheral TG accumulation (Udomkasemsab et al., 2018; Zhang et al., 2020; Li et al., 2023). The study observed increased triglyceride levels in the quercetin and simvastatin groups compared to those in the HFD group. While this increase is not statistically significant, it may be attributed to improved fat metabolism from activating glucose transporter member 4 (GLUT4). Enhanced GLUT4 expression may facilitate lipogenesis, resulting in higher triglyceride levels in rats subjected to HFD (Eseberri et al., 2019; Xia et al., 2024).

Quercetin administration effect on TC levels in plasma of rats induced by HFD

The goal of analyzing the TC profile was to examine how HFD influences plasma cholesterol levels and to investigate the protective role of quercetin in countering these alterations. The study findings in Figure 4 and Table 2 revealed that HFD induction significantly elevated TC levels in rat plasma ($p < 0.01$ and $p < 0.05$). The analysis compared the HFD group with those receiving quercetin to determine its potential in preventing dyslipidemia. A notable elevation in TC was detected in the HFD group compared to the normal chow group, validating the effective induction of dyslipidemia ($p < 0.01$). Subsequent investigation of the HFD + quercetin group revealed diverse results. The HFD plus quercetin 50 mg group did not demonstrate a significant decrease in TC levels compared to the HFD group.

Nonetheless, the HFD + quercetin 100 mg group exhibited a substantial reduction ($p < 0.05$), suggesting its efficacy in reducing cholesterol. The HFD group treated with 200 mg of quercetin exhibited no significant decrease in cholesterol levels. Of the three quercetin doses, only the HFD + quercetin 100 mg group exhibited a statistically significant reduction in cholesterol levels relative to the HFD group ($p < 0.05$). Conversely, the HFD + simvastatin group showed no significant decrease in the TC levels. The data are encapsulated in the subsequent graph (Figure 4).

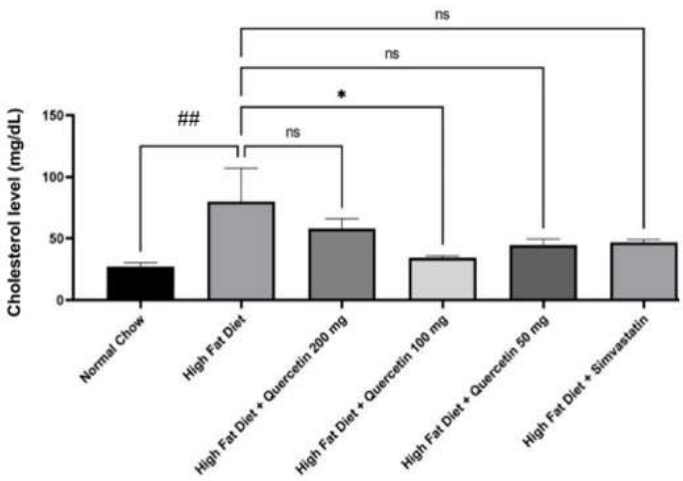

Figure 4. Comparison of total cholesterol profiles among the HFD, treatment, and normal chow groups. The data are presented as mean values ± SEM from 3 to 7 rats in each group. Significance is indicated for the HFD group at *p<0.05. Statistical significance is indicated for the normal chow group (## P < 0.01). One-way ANOVA and Dunnett post-hoc analysis; n = 3 – 7 rats

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An additional dyslipidemia profile noted in this study was TC to assess the preventive potential of quercetin against HFD-induced dyslipidemia. The results indicated lower TC levels in both the quercetin and simvastatin groups than in the HFD group. Palm oil has been used as a fat source to induce increased cholesterol and liver damage in rats (Pehlivanović et al., 2024). The observations showed a notable increase in TC levels. This increase can be associated with the initiation of a HFD that enhances the activity of HMG-CoAR, an essential enzyme in cholesterol synthesis (Shi et al., 2022). The significant decrease ($p < 0.05$) in TC levels noted in the HFD + quercetin 100 mg group may result from the suppression of the AMPK pathway by quercetin, which suppresses the activity of HMG-CoAR, thereby reducing cholesterol production (Wang et al., 2021). While the reduction in cholesterol levels among those taking simvastatin was not statistically significant, it probably resulted from the inhibition of HMG-CoAR (Parihar et al., 2019) (Table 2).

Quercetin administration effect on plasma aspartate transferase levels in rats subjected to HFD

Aspartate aminotransferase (AST) levels were measured to assess hepatic function in rats and evaluate the preventive potential of quercetin. The results of this study, presented in Table 3, indicate that HFD induction did not lead to significant changes in AST levels in rat plasma. This investigation examined the possible protective benefits of quercetin against hepatotoxicity by comparing the HFD group with other treatment groups. An elevation in AST levels was observed in the HFD group compared with that in the normal chow group. However, this difference was not statistically significant. The therapy groups, HFD + quercetin 50 mg and HFD + quercetin 100 mg, exhibited no significant decrease in AST levels compared to the HFD group. The highest dose of the HFD group, 200 mg quercetin, exhibited a minimal increase in AST levels compared to the HFD group. Moreover, the HFD + simvastatin group did not show significantly decreased AST levels compared to the HFD group (Table 3).

Activities of liver enzymes, such as AST and ALT, are critical indicators for assessing liver function. ALT is a precise marker of liver injury, because it is mostly located in the liver (Kathak et al., 2022). This study involved observing the changes in AST and ALT levels to evaluate liver function. The normal chow group exhibited no significant increase in AST levels compared to the HFD group. This insignificance may stem from AST being a less specific indicator of hepatocellular injury (Pehlivanović et al., 2024). The groups administered HFD with quercetin at 50 mg and 100 mg demonstrated reduced AST levels compared with the HFD group. However, the reduction was not statistically significant. This reduction may be linked to the inhibited activity of pro-inflammatory cytokines, such as IL-1β, IL-1, IL-8, and IL-6, which might have a protective effect on the liver (Chen, 2010). The HFD + quercetin 200 mg group exhibited an increase in AST levels, although this change was not significant compared to the HFD group. This increase may be associated with the effect of quercetin on lipid metabolism, particularly gluconeogenesis, through inhibition of the MAPK pathway (Wang et al., 2024). The HFD + simvastatin group showed a slight decrease in AST levels compared to the HFD group, possibly because of the activation of Nrf2, which is recognized for its function in regulating hepatic antioxidant enzymes (Rodrigues et al., 2019).

Quercetin administration affected plasma alanine transferase levels in HFD-fed rats subjected to HFD.

The ALT profile was used to assess liver function in rats, focusing on evaluating the preventive potential of quercetin. The results, illustrated in Figure 5 and Table 4, suggest substantial alterations in ALT levels in the rat plasma. The analysis contrasted the HFD group with other treatment groups to evaluate the efficacy of quercetin in preventing hepatotoxicity. A marked elevation in ALT levels was noted in the HFD group compared to the normal chow group ($p < 0.01$). A significant decrease in ALT levels was noted in the HFD + quercetin 50 mg group compared to that in the HFD group ($p < 0.01$). The HFD + quercetin 100 mg group showed a significant decrease in ALT levels ($p < 0.01$) and capacity to avert hepatotoxicity. A subsequent analysis comparing the HFD group with the HFD plus quercetin 200 mg group demonstrated a substantial reduction ($p <0.01$). All doses of quercetin (50 mg, 100 mg, and 200 mg) demonstrated a significant decrease in ALT levels relative to the HFD group ($p < 0.01$). The HFD + simvastatin group showed a substantially reduced ALT level compared with the HFD group ($p < 0.01$). The subsequent image presents a graphical representation of the analysis results (Figure 5).

The subsequent parameter ALT exhibited substantial elevation in the normal chow group compared to the HFD group, indicating that the induction of HFD caused a hepatotoxic effect on the liver ($p < 0.05$). The elevated ALT levels in the HFD group may be attributed to the hepatic cell damage caused by fat accumulation (Pehlivanović et al., 2024). Observations indicated the preventative efficacy of

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quercetin, as ALT levels were decreased in quercetin-treated groups relative to the HFD group. The reduction in ALT levels may be attributed to quercetin's capacity to block IL-10, thereby reducing several pro-inflammatory cytokines, including TNF-α, that protect liver function (Chen, 2010). A notable reduction in ALT was observed in the HFD + Simvastatin group compared with that in the HFD group. This decrease can be explained by the same process as AST, involving the activation of Nrf2, which is essential for regulating hepatic antioxidant enzymes, rendering ALT a more specific indicator of liver damage (Rodrigues et al., 2019; Pehlivanović et al., 2024). In conclusion, the assessment of liver function, shown by alterations in AST and ALT levels, implies that quercetin has a preventative effect similar to that of simvastatin in reducing the elevation of these enzymes post-HFD induction (Table 4).

Table 2. Table of the effect of quercetin and simvastatin on the levels of cholesterol in rats plasma after induction of HFD feed

Groups	Cholesterol level (mg/dL)
Normal chow	27,00 ± 3,33
HFD	80,00 ± 27,10$^{\#\#}$
HFD + Quercetin 50 mg	44,4 ± 4,92
HFD + Quercetin 100 mg	34,33 ± 1,52*
HFD + Quercetin 200 mg	58,00 ± 8,00
HFD + Simvastatin	46,67 ± 2,32

***K**: Data are presented as the mean ± SEM of 3–7 rats in each group. Significance is indicated for the HFD group at *p<0.05. Statistical significance is indicated for the normal chow group at $^{\#\#}$p<0.01. One-way ANOVA and Dunnett post-hoc analysis; n = 3 – 7 rats

Table 3. Table of the effect of quercetin and simvastatin on aspartate aminotransferase levels in rat plasma after induction of HFD feeding.

Group	Aspartate Aminotransferase (mg/dL)
Normal chow	566,80 ± 65,23
HFD	826,21 ± 50,51
HFD + Quercetin 50 mg	631,05 ± 37,22
HFD + Quercetin 100 mg	753,07 ± 47,69
HFD + Quercetin 200 mg	849,18 ± 94,74
HFD + Simvastatin	768,63 ± 94,78

***K** : The data presented are the mean ± SEM of three rats every group

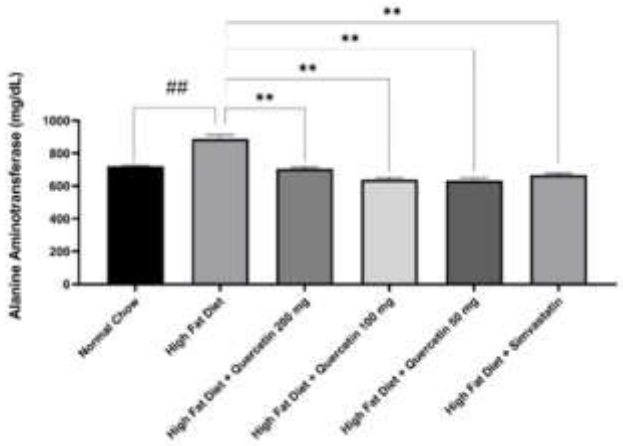

Figure 5 Comparison of ALT profiles among the HFD, treatment, and normal chow groups. The data are presented as mean values ± SEM from three rats in each group. Significance is indicated for the HFD group at *p<0.05 and **p<0.01. Statistical significance is indicated for the normal chow group at $^{\#\#}$p<0.01. One-way ANOVA and Dunnett post-hoc analysis; n = 3 rats

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Table 4. Table of the effect of quercetin and simvastatin on alanine transferase levels in plasma of rats after induction of HFD feed

Group	Alananine Aminotransferase (mg/dL)
Normal chow	720,61 ± 5,08
HFD	887,06 ± 26,75$^{\#\#}$
HFD + Quercetin 50 mg	633,80 ± 12,73**
HFD + Quercetin 100 mg	638,55 ± 12,38**
HFD + Quercetin 200 mg	705,33 ± 10,68**
HFD + Simvastatin	666,00 ± 11,11**

K: Data are presented as the mean ± SEM of three rats in each group. Significance is indicated for the HFD group at *p<0.05 and **p<0.01. Statistical significance is indicated for the normal chow group at $^{\#\#}$p<0.01. One-way ANOVA and Dunnett post-hoc analysis; n = 3 rats

Table 5. Table of the effect of quercetin and simvastatin on HMG-CoAR mRNA in liver rats after induction of HFD feed

Group	Relative Expression of HMG-CoAR mRNA (Fold Change)
Normal chow	0,26 ± 0,02
HFD	1,00 ± 0,62
HFD + Quercetin 50 mg	0,21 ± 0,10
HFD + Quercetin 100 mg	0,38 ± 0,08
HFD + Quercetin 200 mg	9,81 ± 7,92
HFD + Simvastatin	0,09 ± 0,02

K : The data presented are the mean ± SEM of 3 - 7 rats every group

The body regulates cholesterol production through essential enzymes, with HMG-CoAR being crucial for the cholesterol biosynthesis pathway. This process is influenced by the Adenosine Triphosphate-Binding Cassette Transporter (ABCA1), which aids in cholesterol metabolism and HDL formation (Chambers et al., 2019; Shi et al., 2022). HMG-CoAR is the principal target of simvastatin, a commonly prescribed cholesterol-lowering drug that diminishes cholesterol synthesis by blocking this enzyme (Perki, 2022). Quercetin, a natural flavonoid, offers an alternative approach by impacting lipid metabolism through multiple mechanisms, including the inhibition of HMG-CoAR activity via the AMPK pathway (Wang et al., 2021). Furthermore, quercetin augments ABCA1 activity, facilitates cholesterol efflux, and enhancing HDL production (Chambers et al., 2019). This study investigated the comparative expression of HMG-CoAR and ABCA1 mRNA in murine hepatic tissues using PCR. In the HFD group, an increase in relative mRNA expression was observed, although it was not statistically significant, likely due to increased cholesterol synthesis involving HMG-CoAR mRNA expression (Shi et al., 2022). In the HFD + 200 mg quercetin group, a minor yet statistically insignificant increase in HMG-CoAR mRNA expression was observed, potentially caused by the regulation of other enzymes such as LDLR and PCSK9, which are involved in regulating LDL levels (Mbikay et al., 2014). In the HFD combined with quercetin at 50 mg and 100 mg groups, a decrease in HMG-CoAR mRNA expression was observed, although not significant compared to the HFD group, possibly due to the inhibition of HMG-CoAR transcription by quercetin (Wang et al., 2021). The HFD + simvastatin group exhibited a decrease in HMG-CoAR expression, likely attributable to the feedback mechanism of simvastatin influencing other genes associated with liver function, such as SREBP-1C, CYP7A1, and CD36 (Zhang et al., 2020).

Impact of quercetin on ABCA1 mRNA expression rats liver subjected to a HFD

This molecular expression analysis aimed to assess the preventive potential of quercetin in dyslipidemia by comparing it with simvastatin, a widely used cholesterol-lowering drug. The comparative expression of ABCA1 mRNA in the hepatic tissues of rats was assessed using RT-qPCR. The findings in Table 6 demonstrate that HFD induction did not significantly modify the relative expression of ABCA1 mRNA in rat livers. Comparisons between the HFD group and other treatment groups were performed to assess the effect of quercetin at the transcriptional level. The study indicated a decrease in ABCA1 mRNA expression in the HFD group relative to that in the normal chow group, but the decrease was insignificant. The HFD + quercetin 50 mg and HFD + quercetin 200 mg groups exhibited

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negligible increases in ABCA1 mRNA expression compared to the HFD group. Conversely, the HFD + quercetin 100 mg group showed a slight but statistically insignificant decrease in ABCA1 expression. Similarly, the HFD + simvastatin group did not exhibit a significant reduction in ABCA1 mRNA expression compared with the HFD group (Table 6).

ABCA1 is a membrane protein with two transmembrane domains and two nucleotide-binding folds interconnected by an intracellular peptide sequence. Following translation, ABCA1 undergoes glycosylation and is expressed on the cell surface. It mediates the efflux of cellular cholesterol and phospholipids, which requires apolipoproteins in the extracellular space (Wang et al., 2003). In this study, the normal chow group exhibited a marginal, albeit statistically insignificant, reduction in ABCA1 mRNA expression relative to the HFD group. This outcome indicates that HFD induction may not substantially influence ABCA1 gene expression in lipid metabolism but may rather implicate the function of another transporter, ABCG1 (Chambers et al., 2019). The effects of HFD induction, quercetin administration at three different doses, and simvastatin treatment were examined in this study. The HFD + quercetin 200 mg and HFD + quercetin 50 mg groups exhibited elevated ABCA1 mRNA expression, potentially due to stimulation of the AMPK pathway by quercetin, facilitating HDL cholesterol maturation (Chambers et al., 2019; Wang et al., 2021). In contrast, the HFD + quercetin 100 mg group exhibited a reduction in ABCA1 mRNA expression compared with the HFD group, possibly attributable to the phosphorylation of proteins, including p38, TAK1, and MKK3/6, which are capable of modulating ABCA1 expression (Chang et al., 2012). The HFD + simvastatin group showed reduced ABCA1 mRNA expression relative to the HFD group, potentially attributable to feedback processes involving Apolipoprotein A1 (Apo-A1) and ABCG1 (Seeree et al., 2019).

Effect of quercetin administration on liver cellular observations

The histological profile of the liver tissue in experimental animals can be analyzed to evaluate the advancement of dyslipidemia, focusing on cellular alterations. The tissue was processed by creating transverse sections, stained using hematoxylin and eosin (HE), and subsequently examined under a microscope at 200x and 400x magnification (100 µm scale). Figure 6 presents the results of these observations, which were conducted over 36 d. No signs of steatosis or inflammation were observed in the normal-chow group. The HFD group exhibited signs of steatosis. In contrast, the groups that were administered HFD with quercetin at 200 mg/kg BW, 100 mg/kg BW, and 50 mg/kg BW displayed no signs of steatosis or inflammation. Concurrently, data from the HFD + simvastatin cohort indicated evidence of inflammation (Figure 6).

Table 6. Table of the effect of quercetin and simvastatin on ABCA1 mRNA in liver rats after induction of HFD feeding

Group	Relative Expression of ABCA1 mRNA (Fold Change)
Normal chow	0,62 ± 0,38
HFD	0,44 ± 0,41
HFD + Quercetin 50 mg	2,27 ± 2,19
HFD + Quercetin 100 mg	0,30 ± 0,30
HFD + Quercetin 200 mg	10,91 ± 7,60
HFD + Simvastatin	0,10 ± 0,07

K : The data presented are the mean ± SEM of 3 - 7 rats every group

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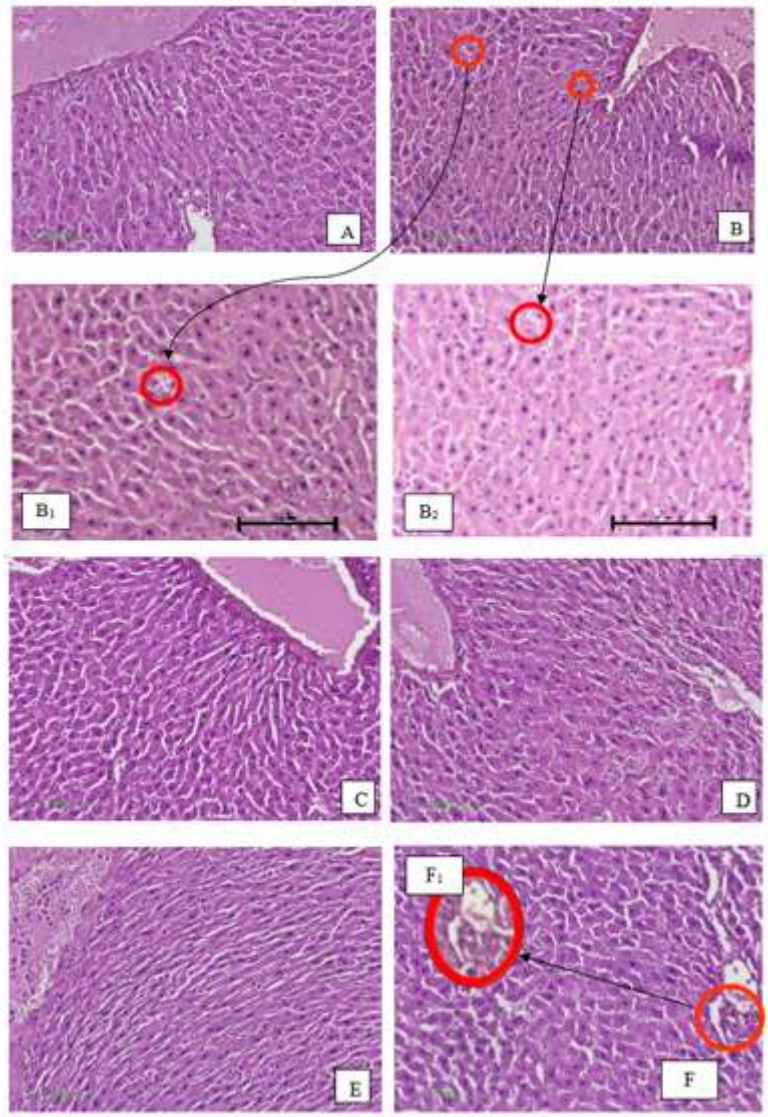

Figure 6. Cross-sections of rat liver at 200x and 400x magnification with hematoxylin and eosin (HE) staining. (Figures 6A) Normal chow group 200x, (Figuress 6B), HFD group, (Figures $6B_1$, $6B_2$) HFD group at 400x magnification, (Figures 6C) HFD + Quercetin 200 mg group at 200x magnification, (Figures 6D) HFD + Quercetin 100 mg group at 200x magnification, (Figures 6E) HFD + Quercetin 50 mg group at 200x magnification and (Figures 6F) HFD + Simvastatin group at 200x magnification, (Figures $6F_1$) HFD + Simvastatin group at 400x magnification. Rat liver tissue was stained with hematoxylin and eosin (HE) staining. Red circles indicate hepatocyte cell abnormalities in the form of steatosis and inflammation. The images are representative of each group. The magnification scale used shows 100 µm and 50 µm

The liver is essential for digestion and metabolism, as a storage site for fat-soluble vitamins, and for controlling cholesterol levels (Kalra et al., 2023). Under hyperlipidemic conditions, changes occur in the blood vessel structure and various tissues, resulting in stiffer tendons and alterations in tissue morphology and cell types (Hill et al., 2024). In this study, we analyzed liver histopathology to detect alterations in hepatic cells. Observations indicated the presence of steatosis in the HFD induction group and inflammation in the HFD + simvastatin group. Increased absorption of fatty acids from the plasma is a primary contributor to liver steatosis. This absorption activates neutrophil-derived proteins, such as proteinase 3, caspase 1, and caspase 11, which further exacerbate steatosis in the liver (Herrero-Cervera et al., 2022; Abo-Zaid OA et al., 2023). The findings for the three quercetin dosages (50, 100, and 200 mg/kg BW) showed no indications of steatosis or inflammation, suggesting a protective effect of quercetin against hepatic cellular injury. The findings for the three quercetin dosages (50, 100, and 200 mg/kg BW) showed no indications of steatosis or

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inflammation, suggesting a protective effect of quercetin against hepatic cellular damage. This protective mechanism may involve the ability of quercetin to induce autophagy in the liver of rats (Cao et al., 2023). In contrast, the HFD + simvastatin group demonstrated inflammation marked by neutrophil infiltration, presumably resulting from excessive buildup of toxic agents in hepatocytes, including free fatty acids and reactive oxygen species, typically linked to NAFLD (Herrero-Cervera et al., 2016; Herrero-Cervera et al., 2022). Additionally, inflammation may result from the side effects of statin use, which are often cited as a reason for discontinuing therapy (Averbukh et al., 2022).

Overall, these findings suggest that quercetin could serve as an alternative therapy for dyslipidemia by improving fat metabolism, particularly TC levels. These doses also affected gene expression and other markers. The doses also affected gene expression; 200 mg/kg increased ABCA1 gene expression, while 100 mg/kg decreased HMG CoAR expression. However, 50 mg/kg could be beneficial for both markers. Additionally, unlike simvastatin, quercetin exhibited low hepatotoxic effects, as indicated by ALT measurements and cellular observations of liver cells. A limitation of this study was the potential gap between the experimental model and its translational relevance to human dyslipidemia. Future studies should explore additional biomarkers relevant to lipid metabolism, such as LXR, SREBP, and ABCG1, to gain a more comprehensive understanding of the effects of quercetin.

CONCLUSION

The administration of quercetin may influence cholesterol metabolism via the AMPK pathway by decreasing the relative expression of HMG-CoAR mRNA at doses of 100 mg and 50 mg, while increasing the relative expression of mRNA ABCA1 at doses of 200 mg and 50 mg. Furthermore, quercetin treatment enhanced the dyslipidemia profile, as shown by the decreased total cholesterol levels. Unlike simvastatin, quercetin also improves liver function under hepatotoxic conditions, as evidenced by reduced alanine transaminase levels and cellular observations.

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AUTHOR CONTRIBUTIONS

Conceptualization, I.AP., S.W., H.D.M., Y.A.P., C.A., J.K.; Methodology, I.AP., S.W., H.D.M., Y.A.P., C.A., J.K.; Software, I.AP., S.W., H.D.M., Y.A.P., C.A., J.K.; Validation, I.AP., S.W., H.D.M., Y.A.P., C.A., J.K.; Formal Analysis, I.AP., S.W., H.D.M., Y.A.P., C.A., J.K.; Investigation, F.W I.AP., S.W., H.D.M., Y.A.P., C.A., J.K.; Resources, I.AP., S.W., H.D.M., Y.A.P., C.A., J.K.; Data Curration; I.AP., S.W., H.D.M., Y.A.P., C.A., J.K.; Writing - Original Draft, I.AP., S.W., H.D.M., Y.A.P., C.A., J.K.; Writing - Review & Editing, I.AP., S.W., H.D.M., Y.A.P., C.A., J.K.; Visualization, I.AP., S.W., H.D.M., Y.A.P., C.A., J.K.; Supervision, I.AP., S.W., H.D.M., Y.A.P., C.A., J.K.; Project Administration, I.AP., S.W., H.D.M., Y.A.P., C.A., J.K.; Funding Acquisition, I.AP., S.W., H.D.M., Y.A.P., C.A., J.K.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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