ORIGINAL ARTICLE

The effects of quercetin on lipid profile, inflammatory biomarkers and ACE2 in a dyslipidaemic rabbit model

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ABSTRACT

Introduction: Dyslipidaemia is a major cardiovascular risk factor associated with elevated low-density lipoprotein (LDL) cholesterol and triglyceride levels. While statins are the primary treatment, inflammation remains a significant predictor of cardiovascular events, highlighting the need for adjunctive therapies targeting both lipids and inflammation. Quercetin, a flavonoid with antioxidant and anti-inflammatory effects, has shown promise in preclinical models, although clinical results are mixed. This study investigates the effects of quercetin on lipid profiles, inflammatory biomarkers (bradykinin, C-reactive protein [CRP], interleukin-6 [IL-6]) and angiotensin-converting enzyme 2 (ACE2) levels in a rabbit model of dyslipidaemia, exploring its potential as an adjunctive therapy for dyslipidaemia.

Materials and Methods: Twenty male New Zealand White rabbits were randomly assigned to four groups (n=4): control, high-fat diet (HFD), HFD with quercetin (20 mg/kg/day), and HFD with atorvastatin (0.43 mg/kg/day, positive control). Rabbits received either a standard or HFD (1.2% cholesterol, 10% coconut oil) alone or HFD with quercetin or atorvastatin co-administered orally for 12 weeks. Blood samples were collected pre- and post-treatment for serum analysis. Body weight was measured weekly, and serum lipid profiles (total cholesterol, triglycerides, high-density lipoprotein [HDL], and LDL) were measured post-treatment. Serum levels of bradykinin, IL-6, CRP, and ACE2 pre- and post-treatment were quantified using enzyme-linked immunosorbent assay.

Results: The HFD significantly increased body weight, total cholesterol, triglycerides, and LDL cholesterol in rabbits, while atorvastatin and quercetin co-treatments effectively reduced total and LDL cholesterol levels ($p < 0.05$) but had no impact on body weight. Neither HFD nor treatments significantly altered HDL cholesterol, bradykinin, IL-6, CRP, or ACE2 levels after 12 weeks. Changes in these inflammatory and enzymatic markers from pre-treatment to post-treatment were also not significant across groups.

Conclusion: Quercetin demonstrated lipid-lowering effects, particularly on total and LDL cholesterol, in a rabbit model of dyslipidaemia. However, it did not significantly affect systemic inflammatory or enzymatic markers. These findings suggest potential lipid-lowering effects independent of inflammation. While quercetin was not superior to atorvastatin, it shows promise as a natural adjunct or alternative therapy.

KEYWORDS:
Quercetin, hyperlipidaemia, bradykinin, inflammation, flavonoid

INTRODUCTION

Dyslipidaemia is a well-established risk factor for cardiovascular disease, often identified only after patients experience acute vascular complications such as ischaemic heart disease or stroke. The association between high cholesterol levels and an increased risk of cardiovascular events is primarily attributed to elevated low-density lipoprotein (LDL) cholesterol.[1] High-density lipoprotein (HDL) cholesterol, initially thought to be inversely related to coronary heart disease (CHD) mortality[2], has since been shown to have more complex associations.[3,4] Furthermore, triglyceride (TG) reduction is linked to a decreased risk of major vascular events in randomised controlled trials; however, this effect is weaker than that of LDL cholesterol.[5]

Current guidelines for managing dyslipidaemia emphasise lowering LDL cholesterol levels as a central strategy, supported by the introduction of new therapeutic agents. Statins remain the first-line treatment for LDL cholesterol reduction in a broad population, including those undergoing primary or secondary prevention. However, for some patients, achieving LDL cholesterol targets may necessitate combination therapy.[6]

While the link between inflammation and atherosclerosis is well-documented[7], the association between systemic inflammatory indices and hyperlipidaemia has been observed but remains insufficiently supported by evidence

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(8). Recent analyses of randomised controlled trials revealed that, among statin-treated patients, inflammation—as measured by high-sensitivity C-reactive protein (CRP)—is a stronger predictor of future cardiovascular events and mortality than cholesterol levels assessed by LDL cholesterol. These findings underscore the importance of exploring adjunctive therapies beyond statins and suggest that combined lipid-lowering and anti-inflammatory treatments may further reduce atherosclerotic risk.[9]

Flavonoids, plant-derived polyphenolic compounds, have gained attention due to their dual antioxidant and anti-inflammatory properties. Quercetin, a flavonoid-rich phytochemical found in foods such as tea, broccoli, onions, papaya and Parkia speciosa,[10-12] has shown promise in preclinical studies. While preclinical studies in rat models of metabolic disease have demonstrated the beneficial effects of synthetic quercetin on diabetes and dyslipidaemia,[13-15] clinical findings remain inconsistent. For instance, a double-blind randomised trial involving women with type 2 diabetes demonstrated that ten weeks of quercetin supplementation significantly reduced systolic blood pressure but had no significant effect on lipid profiles or inflammatory markers (TNF-α, hs-CRP, interleukin-6 [IL-6]).[16] Similarly, another randomised trial in post-myocardial infarction patients found that eight weeks of quercetin supplementation increased total antioxidant capacity but failed to impact inflammatory markers or blood pressure.[17] The limitations of previous clinical studies such as small sample sizes and varying disease contexts underscore the need for more targeted research specifically focused on dyslipidaemia. Animal models provide a standardised disease context, allowing greater experimental control and reproducibility. Among these, rabbits are considered the most suitable model for human dyslipidemia and atherosclerotic disease because they possess cholesteryl ester transfer protein, which is absent in rats and mice. Moreover, rabbits share key similarities with humans in lipoprotein metabolism and demonstrate heightened sensitivity to high-cholesterol diets, making them invaluable for translational research on dyslipidaemia and atherosclerosis.[18] Therefore, the use of rabbit models offers distinct advantages for investigating lipid-related disorders. Such research could yield important insights into the therapeutic potential of quercetin as an adjunctive treatment for dyslipidaemia and its associated inflammatory processes.

This study aimed to evaluate the effects of quercetin on serum lipid profile as well as the inflammatory biomarkers specifically bradykinin, CRP, and IL-6 in a rabbit model of dyslipidemia. Additionally, this study investigates the levels of angiotensin-converting enzyme 2 (ACE2), a cardiovascular protective enzyme known to metabolise the bradykinin derivative des-arg9-bradykinin, within this dyslipidaemic model. It was hypothesised that quercetin could potentially serve as a natural adjunct to conventional lipid-lowering therapy due to its anti-inflammatory properties.

MATERIALS AND METHODS

Quercetin hydrate was purchased from the manufacturer (Merck KGaA, Germany). Atorvastatin (Lipitor®) was purchased from pharmacy, marketed by the pharmaceutical company (Pfizer Inc., USA). A commercial rabbit diet pellet (Gold coin, Malaysia), cholesterol (Pharmaceutical Secondary STD) and hydrogenated coconut oil (MELBIO, Malaysia) were used in this study.

Experimental Animals and Study Design

The sample size was determined using the 'resource equation' method, where the degree of freedom (E) in the analysis of variance (ANOVA) should range from 10 to 20.[19] E = (Total number of animals) – (Total number of groups) = (4 animals per group x 4) - 4 = 12. Since E falls within the acceptable range, and given the use of a medium-sized animal with no mortality observed in this study, a sample size of n = 4 is considered justified. After one week of acclimatization, 20 male New Zealand White rabbits weighing 2.2 kg to 2.4 kg were randomly allocated to four groups, each containing four rabbits (n=4): control, high fat diet (HFD), HFD with concurrent treatment of 20 mg/kg body weight/day quercetin (HFDQ), and HFD with concurrent treatment of 0.43 mg/kg body weight/day atorvastatin (HFDA). Atorvastatin served as the positive control. Except for the control group, which received a standard rabbit diet, the rabbits in the other three groups were fed an HFD consisting of a standard diet supplemented with 1.2% cholesterol and 10% coconut oil for 12 weeks. This dietary regimen and its duration fall within the range of HFDs employed in many studies using hamsters—another well-established hyperlipidaemic animal model with lipid metabolism comparable to that of rabbits and humans.[20] Quercetin and atorvastatin were administered orally, directly via a 1-mL syringe to their respective groups as co-treatments alongside the HFD (abbreviated as HFDQ and HFDA, respectively) during this period. The quercetin dose was selected based on a previous study by Milenković et al.,[21] while the atorvastatin dose was calculated using the animal equivalent dose from a standard human dosage for atorvastatin. Normal saline and corn oil were used as vehicles for atorvastatin and quercetin, respectively. All the animals were provided with 120 g of diet per day and kept under a circadian rhythm of 12 hours of light and 12 hours of dark with free access to drinking water. This study was approved by the Universiti Sultan Zainal Abidin (UniSZA) Animal and Plant Research Ethics Committee (UAPREC/07/011).

Blood Collection and Serum Preparation

Blood samples were withdrawn from the ears or legs after overnight fasting, both before the treatment period (pre-treatment) and at the end of the experiment (post-treatment). All blood samples were centrifuged at 4 °C for 10 minutes at 3000 g. Serum (the supernatants) was collected and stored at –80 °C until further biochemical analysis (lipid profile, inflammatory biomarkers and ACE2). At the end of the experiment, the rabbits were euthanised via carbon dioxide inhalation.

Body Weight Monitoring

Body weight was measured weekly using a calibrated digital weighing machine (Tanita, Japan).

Serum Lipid Profile

Serum was sent to the Innoquest Pathology (formerly Gribbles Pathology) laboratory for analysis of serum total cholesterol (TC), TGs, HDL, LDL, and non-HDL cholesterol using the colourimetric test method principle. The technical

personnel at Innoquest Pathology were blinded to the group allocations.

Serum Inflammatory Biomarkers and ACE2 Quantification
Serum was sent to the Biomics Solution laboratory for analysis. The levels of serum bradykinin, IL-6, CRP and ACE2 were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, Houston, TX, United States) as per the manufacturer's protocol. Each sample was run in duplicate for technical replication. The technical personnel at Biomics Solution were blinded to the group allocations.

Statistical Analysis
Data were tested for normality using the Shapiro–Wilk test. Normally distributed data were analysed using parametric tests, while non-normally distributed data were analysed using non-parametric tests. For the body weight measurements, which were normally distributed, one-way ANOVA was used to assess differences among groups across time points, followed by the Tukey HSD post-hoc test for multiple comparisons. Data were presented as mean ± SD for parametric variables. The blood parameters were not normally distributed and therefore were analysed using non-parametric tests. For lipid profile parameters—TC, TG, HDL, LDL and non-HDL cholesterol—comparisons among the four experimental groups (Control, HFD, HFDQ and HFDA) were made using the Kruskal–Wallis H test. When significant differences were found ($p<0.05$), pairwise comparisons were conducted using the Mann–Whitney U test. For inflammatory and vasoactive markers—IL-6, CRP, bradykinin, and ACE2—within-group (pre vs. post) changes were analysed using the Wilcoxon signed-rank test, while between-group differences in change values (Δ = post – pre) were analysed using the Kruskal–Wallis test. Data were presented as median (interquartile range [Q1–Q3]) for non-parametric variables. A value of $p<0.05$ was considered statistically significant. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software version 24.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

Body weight
There was an increase in body weight among rabbits in all groups throughout the study period (Figure 1). The HFD group showed a significantly higher body weight compared to the control group from week 4 to week 9 (p = 0.004–0.048). Co-treatment with atorvastatin or quercetin did not prevent this weight gain. However, the difference in body weight between the HFDA and the control group was not significant. Body weight was significantly higher in the HFDQ group compared to the control group. There was no significant difference between the HFDA and HFDQ groups.

Serum Lipid Profile
Descriptive analysis, presented as median (interquartile range [Q1–Q3]) values of serum TC, LDL, and non-HDL cholesterol across treatment groups, together with inferential statistics for group comparisons, are shown in Table I. The Kruskal–Wallis test showed significant group differences for TC (p=0.005), LDL (p=0.004), and non-HDL cholesterol (p=0.004), while TG (p=0.068) and HDL (p=0.254) were not significantly different among groups (Table I).

Post-hoc pairwise comparisons using the Mann–Whitney U test showed that the HFD group had significantly higher TC (p=0.021), LDL (p=0.021), and non-HDL (p=0.020) levels compared with the Control group. Both HFDQ and HFDA groups demonstrated significantly lower TC (p=0.021), LDL (p=0.021), and non-HDL (p=0.020) levels compared with the HFD group. No significant difference was observed between HFDQ and HFDA in TC (p=0.248), LDL (p=0.083) and non-HDL (p = 0.083) (Table II).

Serum Inflammatory Markers and Vasoactive Markers (IL-6, CRP, Bradykinin, ACE2)
Within-group comparisons using the Wilcoxon signed-rank test showed no significant pre–post changes in serum bradykinin, ACE2, CRP, or IL-6 levels in any of the groups (p = 0.068–1.44). Descriptive data for each group is presented as median (interquartile range [Q1–Q3]) (Table III).

Between-group comparisons of the change values (Δ = post – pre) using the Kruskal–Wallis H test also showed no significant differences for bradykinin (p = 0.362), ACE2 (p = 0.803), CRP (p = 0.750), or IL-6 (p = 0.907). Descriptive statistics for the change values are expressed as median (interquartile range [Q1–Q3]) (Table IV).

DISCUSSION

In this study, a dyslipidaemic model was successfully established, evidenced by elevated TC and LDL levels induced by an HFD. Elevated LDL cholesterol is an established key driver of cardiovascular risk.[1] Meanwhile, HDL-C cholesterol, once thought to lower CHD mortality [2], has more complex effects. For example, specific HDL subfractions have been linked to a higher risk of CHD [3], and genetic polymorphisms associated with LDL cholesterol levels have been implicated in the risk of myocardial infarction.[4] These findings challenge the simplistic notion that uniformly raising HDL cholesterol levels will consistently reduce CHD risk. Our findings on HDL cholesterol levels also did not demonstrate any significant differences among the groups. Although elevated TG levels are associated with high carbohydrate intake, any dietary practices or factors that lead to excess body weight are associated with elevated TG levels.[22] Hence, the elevated TG levels after HFD in rabbits may be linked to their excess body weight.

Previous research has shown that New Zealand White rabbits can develop hyperlipidaemia within five weeks of HFD exposure and progress to hyperlipidaemia with insulin resistance after ten weeks. Concurrently, HFD has been reported to activate inflammatory pathways in peripheral blood mononuclear cells (PBMCs) as early as five weeks, marked by increased expression of IL-1β and mitogen-activated protein kinase pathways. After prolonged HFD exposure, altered autophagy was observed, indicated by increased biomarkers of autophagy in PBMCs.[23] Autophagy plays a critical role in clearing misfolded proteins and damaged organelles and is known to regulate excessive inflammation.[24] It is plausible that the rabbits in this study

Table I: Serum lipid profile of all groups (median) and differences among the groups

Parameter	Group values (Median [Q1–Q3])				Kruskal–Wallis H test		
	Control	HFD	HFDQ	HFDA	χ^2 (Chi-square)	df	p-value
TC (mmol/L)	2.75 (2.37–3.12)	4.92 (4.75–5.13)	3.90 (3.52–4.27)	3.50 (3.15–3.77)	12.640	3	0.005*
TG (mmol/L)	0.56 (0.41–0.76)	1.03 (0.84–1.21)	0.74 (0.63–0.78)	0.53 (0.42–0.87)	7.123	3	0.068
HDL (mmol/L)	0.71 (0.63–0.86)	0.79 (0.71–0.89)	0.85 (0.75–0.93)	0.88 (0.81–1.03)	4.066	3	0.254
LDL (mmol/L)	1.73 (1.41–1.79)	3.79 (3.71–4.00)	2.72 (2.46–2.98)	2.27 (2.08–2.51)	13.500	3	0.004*
Non-HDL (mmol/L)	1.93 (1.72–2.12)	4.25 (4.14–4.27)	3.06 (2.76–3.35)	2.51 (2.28–2.91)	13.520	3	0.004*

Descriptive analysis was presented as median (interquartile range [Q1–Q3]). Statistical comparisons were performed using the Kruskal–Wallis H test. *Significant at $p < 0.05$.

Table II: Pairwise comparison of serum lipid profile between groups

Parameter	Pairwise Comparison	Mann-Whitney U test	Z	p-value
TC	Control vs HFD	0.000	-2.309	0.021*
	Control vs HFDQ	0.000	-2.309	0.021*
	Control vs HFDA	0.000	-2.021	0.043*
	HFD vs HFDQ	0.000	-2.309	0.021*
	HFD vs HFDA	0.000	-2.309	0.021*
	HFDQ vs HFDA	4.000	-1.155	0.248
LDL	Control vs HFD	0.000	-2.309	0.021*
	Control vs HFDQ	0.000	-2.309	0.021*
	Control vs HFDA	0.000	-2.309	0.021*
	HFD vs HFDQ	0.000	-2.309	0.021*
	HFD vs HFDA	0.000	-2.309	0.021*
	HFDQ vs HFDA	2.000	-1.732	0.083
Non-HDL	Control vs HFD	0.000	-2.323	0.020*
	Control vs HFDQ	0.000	-2.309	0.021*
	Control vs HFDA	0.000	-2.309	0.021*
	HFD vs HFDQ	0.000	-2.323	0.020*
	HFD vs HFDA	0.000	-2.323	0.020*
	HFDQ vs HFDA	2.000	-1.732	0.083

Pairwise comparisons were conducted using the Mann–Whitney U test (two-tailed). *Significant at $p < 0.05$. HFD = high-fat-diet group; HFDQ = high-fat-diet + quercetin; HFDA = high-fat-diet + atorvastatin.

Table III: Within-group comparisons in serum bradykinin, ACE2, CRP and IL-6 between pre- and post- treatment

Parameter	Group	Group values (Median [Q1–Q3])		Wilcoxon signed-rank test	
		Pre-treatment	Post-treatment	Z	p-value
Bradykinin (ng/mL)	Control	6.45 (5.99–7.40)	7.06 (6.02–9.25)	0.000	1.000
	HFD	7.46 (5.03–9.24)	11.64 (5.18–18.40)	1.095	0.273
	HFDQ	5.92 (4.54–7.68)	9.49 (6.21–14.32)	-1.826	0.068
	HFDA	7.37 (6.28–9.04)	9.22 (8.29–12.68)	-1.826	0.068
ACE2 (ng/mL)	Control	46.06 (42.57–49.26)	46.12 (41.07–51.08)	-0.365	0.715
	HFD	44.88 (37.72–49.32)	44.77 (36.36–60.56)	-0.365	0.715
	HFDQ	41.74 (35.00–50.75)	48.97 (35.41–56.85)	-1.461	1.44
	HFDA	50.61 (45.17–52.34)	50.00 (40.20–54.73)	0.000	1.00
CRP (ng/mL)	Control	1167.33 (1144.83–1342.33)	1156.50 (1026.50–1444.00)	0.000	0.715
	HFD	1225.66 (1170.66–1353.16)	1391.50 (1252.75–1504.00)	-1.095	0.273
	HFDQ	1184.00 (1005.66–1289.83)	1184.83 (990.25–1335.66)	0.000	1.000
	HFDA	1212.33 (1118.58–1409.83)	1217.33 (1088.16–1341.50)	0.000	1.000
IL-6 (ng/mL)	Control	266.07 (204.12–315.36)	205.82 (182.29–245.13)	-1.461	1.44
	HFD	263.34 (182.99–296.27)	218.47 (185.13–226.01)	-1.461	1.44
	HFDQ	249.91 (203.32–316.95)	190.36 (164.68–233.77)	-1.826	0.068
	HFDA	249.00 (215.13–299.91)	192.18 (180.82–233.54)	-1.461	1.44

Values are expressed as median (interquartile range [Q1–Q3]). Statistical comparisons between pre- and post-treatment were performed using the Wilcoxon signed-rank test (two-related samples). Significant at $p < 0.05$. HFD = high-fat-diet group; HFDQ = high-fat-diet + quercetin; HFDA = high-fat-diet + atorvastatin.

Table IV: Between-group comparisons of the change values (Δ = post – pre)

Parameter	Group values (Median [Q1–Q3])				Kruskal–Wallis H test		
	Control	HFD	HFDQ	HFDA	χ² (Chi-square)	df	p-value
Δ Bradykinin (ng/mL)	-0.08 (-0.50–3.06)	4.33 (-1.09–10.25)	3.57 (1.66–6.63)	2.03 (1.63–3.83)	3.199	3	0.362
Δ ACE2 (ng/mL)	1.69 (-6.68–5.36)	0.48 (-1.96–11.23)	3.82 (0.41–9.50)	0.22 (-12.14–8.7)	0.993	3	0.803
Δ CRP (ng/mL)	-27.50 (-276.66–276.66)	165.83 (-77.91–310.83)	48.33 (-183.75–166.66)	37.50 (-321.66–190.41)	1.213	3	0.750
Δ IL-6 (ng/mL)	-49.72 (-103.37–0.78)	-44.87 (-70.26–2.14)	-52.73 (-123.41– [-5.22])	-44.54 (-116.13–3.18)	0.551	3	0.907

Values are expressed as median (interquartile range [Q1–Q3]). Statistical comparisons among groups were performed using the Kruskal–Wallis H test. Significant at $p < 0.05$.

experienced early-stage weight gain and dyslipidaemia from the 4th to the 9th weeks. From the 10th week onwards, their weight was not significantly higher than that of the control group, suggesting a possible progression to hyperlipidaemia with insulin resistance. However, this remains speculative as insulin resistance was not assessed. Quercetin did not significantly reduce body weight or serum TG levels in this study, aligning with previous findings that demonstrate variable effects of quercetin on TGs.[14-16] This variability underscores the complexity of lipid metabolism and suggests that quercetin may primarily target LDL cholesterol metabolism rather than TGs. Further investigation is needed to elucidate the specific mechanisms by which quercetin differentially affects lipid fractions.

In contrast, atorvastatin, a well-known statin, demonstrated superior efficacy in lowering both serum TGs and LDL cholesterol levels, as anticipated. Statins achieve their potent lipid-lowering effects by inhibiting HMG-CoA reductase, a key enzyme in cholesterol synthesis.[25] While quercetin showed notable LDL-lowering effects, its comparatively lesser efficacy highlights its potential as an adjunct therapy rather than a standalone treatment for dyslipidaemia. Exploring the potential synergy between quercetin and statins could be a promising avenue for future research, particularly for patients intolerant to high-dose statins or those seeking complementary approaches to enhance lipid control. Additionally, quercetin's natural origin and favourable side-effect profile make it an attractive option for long-term management of dyslipidaemia.

The LDL-lowering effects of unformulated quercetin have been demonstrated in metabolic disease models using rats, typically at doses of 10–100 mg/kg/day [13-15], which corresponds to 5–50 mg/kg/day in rabbits. Studies using rabbits have shown wide range of dosing, from 0.05 mg/kg/day for 12 weeks to 25 mg/kg/day for 90 days, ameliorating hypercholesterolemia.[26,27] Our findings align with these studies.

Moreover, quercetin formulations, such as quercetin: β-cyclodextrin complexes and liposomal encapsulations, have shown enhanced lipid-lowering effects in diabetic rats.[28,29] However, clinical trials have yielded mixed results. For example, in a double-blind randomised trial involving 72 women with type 2 diabetes, 500 mg/day of quercetin capsules for ten weeks significantly reduced systolic blood pressure but had no significant impact on lipid profiles.[16] Several factors may explain quercetin's inconsistent efficacy in clinical studies. Differences in disease stage, such as progression to insulin resistance and the relatively short treatment durations of quercetin treatment, may limit the observed benefits. These findings emphasise the need for further research into optimising quercetin dosage, formulation and timing to enhance its therapeutic efficacy in lipid management.

There are several potential explanations for the lack of elevation in inflammatory markers (IL-6 and CRP) observed in our dyslipidaemia model, despite the presence of metabolic changes. First, it remains unclear whether the activation of inflammatory pathways precedes the development of dyslipidaemia or manifests later in the disease progression following inflammation of the adipose tissue.[30] Second, the inflammatory state in our model might be subtle and subclinical, rendering it undetectable at the protein level. While the interplay between inflammation and atherosclerosis is well-documented [7], evidence linking systemic inflammatory marker levels directly to dyslipidaemia remains limited and inconclusive.[8] Consequently, our study was unable to demonstrate a clear association between systemic inflammation and dyslipidaemia.

Our findings also indicate that quercetin did not significantly influence inflammatory markers in this model. This is consistent with previous clinical studies. For instance, in a double-blind randomised clinical trial involving 72 women with type 2 diabetes, ten weeks of quercetin supplementation (500 mg/day) significantly reduced systolic blood pressure but showed no significant effect on inflammatory biomarkers such as TNF-α, hs-CRP, and IL-6.[16] Similarly, another randomised double-blind, placebo-controlled trial in 88 post-myocardial infarction patients found that eight weeks of quercetin supplementation significantly improved total antioxidant capacity but failed to show any significant effects on inflammatory markers or blood pressure.[17] In contrast, quercetin has been shown to have anti-inflammatory properties.[12] Several factors could explain why quercetin had no significant effect on inflammation in these clinical studies as well as in our study. First, the anti-inflammatory activity of quercetin may not be exerted in the absence of a significant inflammatory state, as observed in our study. Second, differences in disease stages, such as insulin

resistance or post-myocardial infarction in the aforementioned clinical studies, likely influenced the outcomes, as these conditions involve advanced metabolic and inflammatory complications. Thirdly, this model is optimal for dyslipidaemia but may have limitations in representing inflammation. The lack of systemic inflammation in the model could be a key limitation in testing anti-inflammatory potential. Moreover, the relatively short durations of supplementation (8–10 weeks) may have been insufficient to produce measurable changes in inflammatory markers. These findings underscore the complexity of quercetin's effects on inflammation and highlight the need for further studies to optimise its dosage, formulation, and treatment duration for effectively targeting inflammation in dyslipidaemia and related conditions.

This study investigates the effects of quercetin on bradykinin, a relatively understudied inflammatory biomarker. Bradykinin, often referred to as a 'proinflammatory kinin', plays a pivotal role in various inflammatory conditions (31). Bradykinin is a nonapeptide derived from high-molecular-weight kininogen, and elevated levels beyond physiological thresholds in cardiometabolic diseases can have harmful effects. For instance, Perhal et al.[32] demonstrated that transgenic overexpression of B2R in a hypercholesterolemic atherosclerotic mouse model exacerbated atherosclerotic plaque formation. In this study, bradykinin levels did not significantly differ between pre- and post-treatment or across groups, possibly because bradykinin plays a limited role in subclinical, non-infectious inflammation and its short half-life leads to rapid degradation, reducing measurable changes. It has been suggested that in a physiological state, an increase in des-Arg9-bradykinin (DABK) in cardiometabolic patients acts as a compensatory response to elevated blood pressure.[7]

Bradykinin is closely linked to ACE2, which degrades DABK into inactive metabolites.[33] Clinical and in vivo studies have reported that hypertension and metabolic syndrome are associated with elevated ACE2 levels.[34,35] As part of the protective arm of the renin-angiotensin system (RAS), ACE2 has demonstrated anti-atherosclerotic effects in numerous in vivo studies.[36,37] Elevated ACE2 levels in patients with cardiometabolic diseases and risk factors suggest a compensatory role in CHD [38] and indicate its potential as a biomarker for cardiovascular risk assessment before disease onset. In this study, ACE2 levels showed no significant changes across groups or between pre- and post-treatment. The lack of significant changes in ACE2 levels may also be attributed to factors such as the treatment duration, or the change might be subtle, rendering it undetectable at the protein level.

There are limited primary study investigating the effect of quercetin on bradykinin. While studies are reporting on the inhibitory effect of quercetin on rhACE2 activity in assay conditions [39] and expression of ACE2 in SARS-CoV-2 infection models [40], it has never been substantially investigated in cardiovascular disease models. This study may represent one of the first to investigate ACE2 and bradykinin in a dyslipidaemic rabbit model.

This study has some limitations that should be acknowledged. The small sample size may limit the generalisability of the findings, and larger studies are needed to confirm the results. Additionally, the duration of the study may not have been sufficient to observe the long-term effects of quercetin on ACE2 expression and other cardiovascular outcomes. Finally, the dosage of quercetin used in this study may not have been optimal for eliciting maximal therapeutic effects, and future studies should explore different dosages, formulations and treatment regimens.

CONCLUSION

In summary, this study demonstrated that quercetin significantly improved serum TC and LDL levels, albeit without significant changes in serum IL-6, CRP, bradykinin and ACE2 levels, in a rabbit model of diet-induced dyslipidaemia. While it did not outperform atorvastatin in all measured parameters, quercetin showed potential as a natural alternative or adjunct therapy to conventional lipid-lowering drugs. This further suggests that its lipid-lowering effects may be independent of its anti-inflammatory effects.

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