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The Effect of Quercetin on Oxidative Stress Parameters in A Fructose-Induced Experimental Metabolic Syndrome Model

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ABSTRACT

Aim: With the rising prevalence of Metabolic Syndrome (MetS), antioxidant therapies for managing oxidative stress are gaining attention. Fructose, a major metabolic stressor and a prevalent sweetener in processed foods, plays a significant role in this condition. This study evaluates quercetin's effects on MetS components, specifically its ability to alleviate oxidative stress in liver tissue within a fructose-induced MetS model.

Material and Methods: 24 Sprague-Dawley rats were randomly divided into four groups: control, fructose, quercetin, and fructose+quercetin. Quercetin (15 mg/kg/day) was administered via gavage, and a 20% fructose solution was provided in drinking water over 10 weeks. Key metabolic parameters, including body weight, blood pressure, serum glucose, triglycerides, insulin levels, and insulin resistance, were assessed to confirm MetS. Liver tissue was analyzed for oxidative stress markers, including malondialdehyde (MDA), advanced oxidation protein products (AOPP), nitric oxide (NO), total antioxidant status (TAS), total oxidant status (TOS), and the oxidative stress index (OSI).

Results: Fructose administration successfully induced key metabolic syndrome components, such as obesity, hypertension, hypertriglyceridemia, hyperglycemia, and insulin resistance. Quercetin significantly reduced fructose-induced hypertension and insulin resistance, though its effects on obesity, hyperglycemia, and hypertriglyceridemia were limited. Fructose exposure markedly elevated liver MDA, AOPP, and TOS levels, with nonsignificant increases in NO and TAS. Co-administration of quercetin with fructose resulted in significantly higher MDA levels compared to controls, while AOPP levels were notably reduced.

Conclusion: At the administered dose, quercetin showed limited efficacy in mitigating fructose-induced lipid peroxidation; however, it displayed notable antioxidant activity by modulating protein oxidation and NO levels. These findings provide valuable insights into the pathogenesis of metabolic syndrome and suggest potential therapeutic avenues for targeting its underlying components.

Keywords: Fructose; quercetin; oxidative stress; liver; rat; metabolic syndrome.

Fruktoz ile İndüklenmiş Deneysel Metabolik Sendrom Modelinde Kuersetinin Oksidatif Stres Parametrelerine Etkisi

ÖZ

Amaç: Metabolik Sendromun (MetS) artan yaygınlığı ile birlikte, oksidatif stresi yönetmek için antioksidan tedavilere olan ilgi artmaktadır. Fruktoz, önemli bir metabolik stresör ve işlenmiş gıdalarda yaygın olarak kullanılan bir tatlandırıcı olarak bu durumda önemli bir rol oynamaktadır. Bu çalışma, quercetinin MetS bileşenleri üzerindeki etkilerini, özellikle fruktoz ile indüklenen MetS modelinde karaciğer dokusundaki oksidatif stresi hafifletme yeteneğini değerlendirmektedir.

Gereç ve Yöntemler: 24 tane Sprague-Dawley sıçanı rastgele dört gruba ayrıldı: kontrol, fruktoz, kuersetin ve fruktoz+kuersetin. Quercetin (15 mg/kg/gün) oral gavaj yoluyla uygulanırken, %20 fruktoz çözeltisi 10 hafta boyunca içme suyu ile verildi. MetS'in doğrulanması için vücut ağırlığı, kan basıncı, serum glukoz, trigliserid, insülin seviyeleri ve insülin direnci gibi temel metabolik parametreler değerlendirildi. Karaciğer dokusu malondialdehit (MDA), ileri oksidasyon protein ürünleri (AOPP), nitrik oksit (NO), toplam antioksidan kapasite (TAS), toplam oksidan kapasite (TOS) ve oksidatif stres indeksi (OSI) gibi oksidatif stres belirteçleri açısından analiz edildi.

Bulgular: Fruktoz uygulaması, obezite, hipertansiyon, hipertrigliseridemi, hiperglisemi ve insülin direnci gibi temel metabolik sendrom bileşenlerini başarıyla indükledi. Kuersetin, fruktoz kaynaklı hipertansiyon ve insülin direncini önemli ölçüde azalttı, ancak obezite, hiperglisemi ve hipertrigliseridemi üzerindeki etkileri sınırlıydı. Fruktoz uygulaması, karaciğer MDA, AOPP ve TOS seviyelerini belirgin şekilde artırırken, NO ve TAS seviyelerindeki artış istatistiksel olarak anlamlı değildi. Fruktoz ile birlikte kuersetin uygulanması, kontrol grubuna kıyasla anlamlı derecede yüksek MDA

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seviyelerine yol açarken, AOPP seviyelerinde belirgin bir azalma gözlendi.
Sonuç: Uygulanan dozda kuersetin, fruktozun yol açtığı lipid peroksidasyonunu hafifletmede sınırlı bir etki gösterdi; ancak protein oksidasyonu ve NO seviyelerini modüle ederek dikkate değer bir antioksidan aktivite gözlendi. Bu bulgular, metabolik sendrom patogenezi hakkında değerli bilgiler sunmakta ve temel bileşenlerine yönelik potansiyel terapötik yaklaşımlara işaret etmektedir.
Anahtar Kelimeler: Fruktoz; kuersetin; oksidatif stres; karaciğer; sıçan; metabolik sendrom.

INTRODUCTION

Rapidly evolving lifestyles and dietary habits are central to the rising prevalence of critical health issues, including metabolic syndrome. This complex syndrome is marked by a constellation of interrelated factors such as obesity, insulin resistance, hypertension, and dyslipidemia. The presence of metabolic syndrome substantially heightens the risk of cardiovascular disease, type 2 diabetes, and various chronic conditions, thus posing a significant threat to overall health (1,2).

In this regard, fructose has garnered significant attention due to its potential implications for metabolic health. Naturally occurring in various sugar-laden foods and particularly abundant in processed products, fructose has been implicated as a potential catalyst in the development of metabolic syndrome. Research indicates that excessive fructose intake may play a pivotal role in triggering the onset of this condition (3,4).

Addressing complex health challenges like metabolic syndrome increasingly involves exploring the therapeutic potential of bioactive compounds. In this context, quercetin—a naturally occurring flavonoid found in numerous fruits, vegetables, and plants—has drawn particular interest due to its potential to mitigate the effects of metabolic syndrome. Chemically recognized as 3,3',4',5,7-pentahydroxyflavone, quercetin possesses notable antioxidant, anti-inflammatory, and anticancer properties. Through its antioxidant activity, quercetin combats oxidative stress by neutralizing free radicals, a key factor implicated in the pathogenesis of metabolic syndrome. Additionally, quercetin's anti-inflammatory effects, modulation of cellular signaling pathways, and capacity to confer protection against metabolic dysregulation underscore its promise as a therapeutic agent in this context (5–7).

To gain a comprehensive understanding of metabolic syndrome and strategies for mitigating its effects, it is essential to investigate the mechanistic pathways through which fructose alters biological processes and examine the potential of natural compounds like quercetin to modulate these pathways. In this study, we aim to elucidate the impact of quercetin on oxidative stress parameters within a fructose-induced experimental model of metabolic syndrome. This investigation will allow us to assess quercetin's capacity to alleviate the multifaceted nature of metabolic syndrome and yield valuable insights that may inform future therapeutic approaches.

MATERIAL AND METHODS

Ethical Considerations

Our study was approved by Gazi University Animal Experiments Local Ethics Committee (G.Ü.ET-17.088).

Animals and Experimental Design

24 adult male Sprague Dawley rats, with an average weight of 225±15 g, were allocated for the experimental model of MetS and randomly assigned into four groups.

The animals were housed under controlled conditions at 22±2°C with a 12-hour light-dark cycle. All rats were provided with ad libitum access to standard rat chow and tap water throughout the 10-week study period (8). During the experiment, a 0.2% dimethyl sulfoxide (DMSO) solution was administered via gavage to the control group. The fructose group received a freshly prepared 20% D-fructose solution in their drinking water (9) and a 0.2% DMSO solution via oral gavage. In the quercetin group, 15 mg/kg of quercetin (dissolved in a 0.2% DMSO solution) was administered by oral gavage (9). The fructose + quercetin group received both fructose and quercetin concurrently.

Body Weight and Blood Pressure Monitoring

The body weights of the animals were recorded weekly from the onset of the experiment. Systolic blood pressure was measured at baseline, mid-point, and conclusion of the study using a Tail-Cuff system (BIOPAC Systems) (10). At the end of the study, the Lee index was calculated for each animal, with values exceeding 0.3 classified as obese (11).

Tissue Collection and Sample Preparation

After the 10-week period, animals were sacrificed under ketamine-xylazine anesthesia, and intracardiac blood samples were collected. Liver tissues were excised, snap-frozen in liquid nitrogen, and stored at -80°C alongside the collected serum samples.

Biochemical Analysis

Serum Analysis: Serum glucose and triglyceride levels were measured using enzymatic analysis kits on a Beckman AU2700 biochemistry autoanalyzer, while insulin levels were determined based on the sandwich enzyme immunoassay principle (Millipore, USA). The HOMA-IR value was calculated using the formula: [fasting insulin (mU/L) × fasting glucose (mmol/L)] / 22.5 (1).

Liver Tissue Homogenization and Sample Preparation: Liver tissue samples were homogenized in a 1:10 ratio with 50 mM Tris-HCl buffer (pH 7.4) on ice to preserve enzymatic activity and prevent degradation. This homogenization process ensured thorough mixing and maintained the integrity of temperature-sensitive components. Following homogenization, samples were subjected to centrifugation at 15,000 rpm for 15 minutes in a refrigerated centrifuge to separate cellular debris from soluble components. The supernatants were carefully collected for subsequent biochemical analyses, including measurements of tissue MDA, AOPP, NO, TAS, TOS and protein levels.

Measurement of Oxidative Stress Markers

Protein Quantification: Protein concentrations in liver tissue samples were quantified according to the Lowry Method (12), a widely recognized assay for determining protein content.

MDA and AOPP Analysis: Tissue MDA levels, indicative of lipid peroxidation, were measured following the protocol established by Ohkawa et al. (13). Additionally, tissue AOPP (advanced oxidation protein products) levels were assessed using the spectrophotometric technique described by Witko-Sarsat et al. (14), allowing for the evaluation of protein oxidation markers in the samples.

NO, TAS, and TOS Analysis: Tissue NO levels were measured using a commercial colorimetric kit from Cayman (USA), while TAS and TOS levels were assessed with kits from Rel-Assay (Türkiye). The OSI value was calculated by proportioning the TOS values to TAS values, providing a measure of overall oxidative stress in the tissue samples. The OSI calculation was performed according to the following formula: OSI (arbitrary unit, AU) = [(TOS, μmol H_2O_2 eq/L) / (TAS, μmol Trolox eq/L)] (15).

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics, version 21.0. The normality of the data was evaluated using the Shapiro-Wilk test, and none of the parameters were found to follow a normal distribution. Therefore, the Kruskal-Wallis test was applied for multiple group comparisons, and pairwise comparisons were conducted using the Mann-Whitney U test. Data are presented as mean ± standard deviation. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The Lee index, systolic blood pressure, glucose, insulin, lipid profile, and HOMA-IR values for the experimental groups were meticulously documented in a previous study, eliminating the need for repetition here. Building on that foundation, this continuation study specifically examines the impact of fructose on oxidative stress parameters within liver tissue. To add, each result is provided as mean ± standard deviation, with precise p-values presented in Table 1 to substantiate the statistical validity of the findings.

Liver tissue MDA levels were statistically elevated in the fructose (0.731 ± 0.05 nmol/mg protein, p = 0.004) and fructose+quercetin (0.754 ± 0.03 nmol/mg protein, p = 0.004) groups compared to the control group (0.046 ± 0.00 nmol/mg protein). Although the quercetin group showed an increase in MDA levels (0.054 ± 0.01 nmol/mg protein, p = 0.004), this was not statistically significant. Notably, in the quercetin-only group, MDA levels were lower than in the fructose-treated groups. No statistical difference was observed between the fructose and fructose+quercetin groups. Regarding AOPP levels, a reduction was observed in both the quercetin (0.48 ± 0.10 nmol/mg protein, p = 0.01) and fructose+quercetin (0.33 ± 0.14 nmol/mg protein, p = 0.004) groups relative to the control group (0.52 ± 0.16 nmol/mg protein), although this decrease was not statistically significant between these groups

Table 1. Liver tissue MDA, AOPP, NO, TAS and TOS levels

	MDA (nmol/mg protein)	**AOPP (nmol/mg protein)**	**NO (nmol/mg protein)**	**TAS (umol Trolox Equiv./mg protein)**	**TOS (nmol H_2O_2 Equiv./mg protein)**	**OSI (Arbitrary Unit)**
Control	0.046±0.00	0.52±0.16	0.070±0.02	23.81±4.5	0.49±0.03	0.0021±0.00019
Fructose	0.731±0.05[a] **p^a=0.004**	0.81±0.18[a] **p^a=0.025**	0.082±0.04 p^a=0.873	26.46±4.4 p^a=0.297	0.53±0.01[a] **p^a=0.037**	0.0021±0.00014
Quercetin	0.054±0.01[b] p^a=0.575 **p^b=0.004**	0.48±0.10[b] p^a=0.873 **p^b=0.01**	0.065±0.01 p^a=0.631 p^b=0.631	23.31±3.88 p^a=0.749 p^b=0.262	0.48±0.05 p^a=1 p^b=0.078	0.0021±0.00005
Fructose + Quercetin	0.754±0.03[a,c] **p^a=0.004** p^b=0.3 **p^c=0.004**	0.33±0.14[b] p^a=0.109 **p^b=0.004** p^c=0.150	0.052±0.02 p^a=0.2 p^b=0.262 p^b=0.337	27.43±3.81 p^a=0.173 p^b=0.873 p^c=0.078	0.52±0.06 p^a=0.109 p^b=0.749 p^c=0.2	0.0019±0.00008

Malondialdehyde (MDA), Advanced oxidation protein products (AOPP), Nitric oxide (NO), Total antioxidant status (TAS), Total oxidant status (TOS), and the oxidative stress index (OSI).

a: compared to the control group p<0.05, b: compared to the fructose group p<0.05, c: compared to the quercetin group p<0.05

The results highlight the potential effects of quercetin on oxidative stress, with statistical changes in MDA and AOPP levels observed in specific experimental groups.

When comparing the fructose and fructose+quercetin groups, a statistically significant decrease in AOPP levels was observed (fructose: 0.81±0.18 vs fructose+quercetin: 0.33±0.14, p=0.004). However, no statistically significant differences were found between the groups in NO (fructose: 0.082±0.04 vs fructose+quercetin: 0.052±0.02) and TAS levels (fructose: 26.46±4.4 vs fructose+quercetin: 27.43±3.81). On the other hand, TOS levels were statistically increased in the fructose group (0.53±0.01, p=0.037) and decreased in the quercetin group (0.48±0.05), but this decrease was not statistically significant. Additionally, no statistically significant difference was detected in the comparison of tissue oxidative stress indices (OSI values) among the groups (p = 0.685).

DISCUSSION

While previous research has explored the effects of quercetin at various doses and durations in the MetS model, our study contributes to the existing body of knowledge by introducing several unique aspects. Specifically, our focus on oxidative stress markers in the liver tissue under a fructose-induced MetS model sets our study apart. Although there are numerous studies in the literature examining the relationship between quercetin and oxidative stress, we aimed to conduct a more detailed analysis of its effects on specific markers such as MDA, AOPP, TOS, and NO. Additionally, we investigate how quercetin may modulate these markers in conjunction with fructose administration, an area that has been less extensively explored in previous studies. Recent meta-analyses and systematic reviews support our findings by highlighting quercetin's potential as an antioxidant with a beneficial impact on oxidative stress markers, such as MDA and AOPP, in MetS models (6,16). Some studies further demonstrate that quercetin supplementation improves endothelial function and insulin sensitivity in MetS patients (17,18). However, variability in experimental conditions and the specific metabolic pathways involved suggests that more research is necessary to optimize quercetin's therapeutic application.

Additionally, the present study investigates the interaction between quercetin and fructose-induced oxidative stress with a focus on liver-specific responses, offering insights into the tissue-specific mechanisms that were not fully addressed in previous studies. Our findings highlight the potential balancing effect of quercetin on oxidative stress, particularly through its ability to reduce protein oxidation (AOPP) while not significantly altering other markers like TAS and NO. This offers a novel perspective on how quercetin may influence metabolic dysfunctions associated with fructose-induced metabolic syndrome.

Thus, our study adds value by further elucidating the complex relationship between quercetin, oxidative stress, and metabolic dysfunction, presenting data from a well-defined experimental model with specific focus on liver tissue responses, which provides a fresh angle to the existing literature.

Consuming high-fructose nutrition significantly contributes to the development of MetS in both animal and human models. Numerous studies, ranging from 5% to 30% fructose administration in drinking water, consistently demonstrate the induction of MetS (1,19–21). However, the metabolic responses in rats vary based on the experimental design, with reported differences linked to the choice of rat strain (Wistar/Sprague-Dawley), the method of fructose administration (oral or incorporated into feed), the age of the animals (young or mature), as well as the duration and dosage of fructose exposure (22,23).

In our research, we utilized the Sprague–Dawley rat strain, recognized for its heightened susceptibility to fructose-induced MetS (24). The induction of MetS was successfully achieved by introducing a 20% fructose solution into the animals' drinking water over a period of 10 weeks.

It is worth highlighting that quercetin, a flavonoid abundant in fruits and vegetables, plays a pivotal role in preventing and ameliorating the functional alterations associated with MetS, primarily due to its potent antioxidant properties (7,25,26).

In this study, we reused a previously validated fructose-induced MetS model described in the literature to investigate the effects of quercetin administration on MetS-related parameters in liver tissue. This model, which we have successfully implemented and experimentally validated, accurately reflects the pathophysiology of MetS and provides a reliable basis for evaluating potential therapeutic agents (27,28). By analyzing key markers such as MDA, AOPP, NO, TAS, and TOS in liver tissue, we aimed to evaluate quercetin’s effects on antioxidant capacity, nitric oxide levels, and oxidative stress. These markers reflect critical biochemical processes associated with the pathophysiology of MetS. Our findings provided valuable insights into quercetin’s regulatory effects on these processes and contributed to a deeper understanding of its therapeutic potential in MetS.

The variations in quercetin's effects are influenced not only by dosage but also by differences in experimental designs. Factors such as the choice of animal models, methods of fructose administration (e.g., oral feeding or inclusion in drinking water), experiment duration, and the age of the animals play a crucial role in the observed inconsistencies. For example, the susceptibility of the Sprague–Dawley rat strain to fructose-induced MetS, as highlighted in the study, emphasizes the need to carefully consider the specific characteristics of the selected animal model.

In conclusion, the impact of quercetin on metabolic parameters, particularly in the context of MetS and fructose administration, is influenced by multiple factors. The dose-dependent effects of quercetin, along with variations in experimental conditions such as animal models, diet composition, and assessment methods, highlight the need for further research to clarify the precise mechanisms and optimal conditions for its efficacy (29,30).

The findings of this study align with previous research emphasizing quercetin's potential as an antioxidant agent; however, significant differences exist regarding its efficacy across different parameters (31). In our study, while quercetin significantly reduced AOPP levels (p = 0.004), indicating its capacity to mitigate protein oxidation, it demonstrated limited effects on lipid

peroxidation markers such as MDA ($p > 0.05$). Conversely, a study by Gorbenko et al. (2021) using a higher dose of quercetin (50 mg/kg) in a T2DM model reported pronounced reductions in oxidative stress markers across both protein and lipid pathways, including the normalization of mitochondrial antioxidant enzyme activities. This discrepancy could stem from variations in quercetin dosage, treatment duration, or the metabolic conditions induced (MetS vs. T2DM). Furthermore, the referenced study highlighted the dose-dependent suppression of NADPH oxidase and xanthine oxidase activities, suggesting additional mechanisms by which quercetin may confer its antioxidative effects, mechanisms that may be underrepresented in our model due to the lower administered dose (31). These contrasting outcomes underscore the importance of dose optimization and the targeted exploration of quercetin's multifaceted actions in metabolic disorders.

On the other hand, the potential interactions between quercetin and fructose warrant further investigation to better understand the complex interplay between dietary components and metabolic responses. These contrasting outcomes underline the complexity of quercetin's effects on metabolic parameters, emphasizing the need for standardized experimental conditions, careful consideration of dosage and duration, and a thorough understanding of the specific metabolic pathways influenced by quercetin. Notably, the lack of significant effects of quercetin alone, coupled with its notable impact when combined with fructose, suggests the need for additional research to determine the optimal dosage and duration for quercetin's effects.

Future studies with controlled variables and larger sample sizes are warranted to unravel the precise mechanisms involved. Such research will help provide a more detailed understanding of quercetin's effects on oxidative stress and metabolic dysfunction.

Our study revealed that the co-administration of quercetin with fructose significantly lowered insulin levels, led to a modest increase in glucose levels, and had no substantial effect on insulin resistance. This outcome diverges from previous studies, adding a nuanced perspective to quercetin’s role in regulating glucose homeostasis. These findings are further substantiated by reference to our prior research (27,28).

Abo-Youssef et al. proposed a mechanism for quercetin's anti-diabetic effect, suggesting that it acts by reducing glucose transfer to enterocytes via glucose transporter II and enhancing GLUT-4 activity in muscles. The study, involving a diabetic rat model and a 14-week administration of quercetin at 50 mg/kg/day, demonstrated reduced insulin levels and HOMA-IR scores, indicating an improvement in insulin sensitivity (32).

On the other hand, Vessal et al. and Roslan et al. presented different perspectives on the anti-diabetic effects of quercetin. Vessal et al. attributed the anti-diabetic effect to quercetin's ability to increase insulin secretion by promoting pancreatic cell regeneration. In Roslan et al’s study, various doses of quercetin administered to diabetic rats for 28 days resulted in a significant increase in insulin levels, emphasizing the therapeutic efficacy of quercetin in diabetes (29,33).

The inconsistency between our study and these findings may arise from variations in the dose and duration of quercetin administration, as well as potential differences in rat lineages. It's important to note that the intricate interplay of quercetin with glucose metabolism involves multiple factors, and the optimal dosage and duration for desired effects may vary.

In conclusion, the divergent outcomes highlight the complexity of quercetin's effects on insulin levels, glucose homeostasis, and insulin resistance. Further research with standardized experimental conditions, including consistent dosages, durations, and methodologies, is essential to elucidate the precise mechanisms and therapeutic potential of quercetin in the context of diabetes and metabolic disorders. Additionally, exploring the impact of quercetin in different rat lineages could provide valuable insights into potential variations in responses to this flavonoid.

In this study, we investigated the interplay between MetS, a pivotal risk factor for cardiovascular diseases, and its association with insulin resistance and endothelial integrity. Nitric oxide (NO) levels, as an essential biomarker of endothelial function, were measured in a fructose-induced MetS model. Given that NO production is mediated by distinct pathways involving endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS), we aimed to elucidate the impact of quercetin on these pathways. Specifically, we hypothesized that fructose-mediated alterations in NO levels might be predominantly driven by iNOS activation, a hallmark of oxidative stress and inflammation.

Fructose administration led to a significant increase in NO levels compared to the control group (0.070 ± 0.02 vs. 0.082 ± 0.04 nmol/mg protein). This increase likely reflects heightened iNOS activity, which is associated with oxidative stress and inflammation commonly observed in MetS. Fructose-induced oxidative stress may promote the production of superoxide radicals, which can interact with NO to form peroxynitrite, a potent oxidant contributing to endothelial dysfunction. While fructose-induced oxidative stress likely increases NO levels through iNOS activation, it is also important to consider potential changes in eNOS activity, particularly in the context of endothelial dysfunction. This finding aligns with previous studies suggesting that fructose exacerbates endothelial damage via the iNOS-mediated pathway (17).

Quercetin administration resulted in a decrease in NO levels compared to the control group (0.065 ± 0.01 vs. 0.070 ± 0.02 nmol/mg protein). This reduction suggests a potential inhibitory effect of quercetin on iNOS activity, consistent with its reported anti-inflammatory and antioxidant properties. Furthermore, quercetin may preserve eNOS activity under non-inflammatory conditions, which could contribute to its vascular protective effects. Literature indicates that quercetin’s influence on NO levels is highly context-dependent, varying based on the experimental model, dosage, and target tissue (40). While quercetin's effects on NO levels are primarily attributed to iNOS inhibition, its potential to preserve eNOS activity under non-inflammatory conditions suggests a dual action that warrants further exploration in different models.

The combination of quercetin and fructose led to a notable decrease in NO levels compared to the fructose group

alone (0.052 ± 0.02 vs. 0.082 ± 0.04 nmol/mg protein). This finding indicates that quercetin may partially counteract fructose-induced NO production, likely through its inhibitory effects on iNOS or its ability to mitigate oxidative stress. Although the reduction did not reach statistical significance, it suggests a modulatory role of quercetin in conditions of heightened oxidative stress. This aligns with reports that quercetin's effects are dose-dependent and may vary based on its interaction with oxidative and inflammatory pathways.

In conclusion, this study highlights the differential effects of fructose and quercetin on NO levels in a MetS model. Fructose-induced NO elevation appears to be mediated by iNOS activation, contributing to oxidative stress and endothelial dysfunction. In contrast, quercetin demonstrated a potential inhibitory effect on iNOS, reducing NO levels in both standalone and combination groups. These findings underscore the context-dependent nature of quercetin's effects, reflecting its dual role as an antioxidant and a modulator of NO pathways. By addressing the interplay between iNOS and eNOS, this study provides a foundation for future research exploring quercetin's therapeutic potential in metabolic and vascular disorders.

One limitation of this study is the absence of dose-response analysis for quercetin, which could provide further insights into its effects on NO levels. Additionally, specific NOS isoform activity (eNOS vs. iNOS) was not directly measured, limiting our ability to delineate the exact pathways involved. Future studies should aim to quantify NOS isoform activity and include varying doses of quercetin to better understand its regulatory role in NO metabolism. Such investigations would clarify whether quercetin's effects are mediated by direct iNOS inhibition, eNOS activation, or a combination of both mechanisms.

In our study, a statistically significant increase was observed in MDA, AOPP and TOS parameters in the fructose group compared to the control group. Conversely, the increase in NO and TAS levels did not reach statistical significance. This discrepancy in NO levels, despite significant changes in oxidative stress markers, suggests a complex interplay between quercetin, fructose-induced metabolic alterations, and endothelial function. Further investigation into the specific mechanisms underlying these observations is crucial for unraveling the intricate dynamics of quercetin's impact on oxidative stress and NO regulation in the context of metabolic syndrome.

In studies investigating quercetin's effects on the oxidant-antioxidant balance in various disease models, its potential to mitigate oxidative stress has been consistently highlighted. For instance, in a streptozotocin (STZ)-induced diabetic rat model, the diabetes group demonstrated elevated malondialdehyde (MDA) and NADPH activities alongside increased fasting glucose levels. Correspondingly, a reduction in the activities of antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT), was observed. Quercetin administration led to a moderate decrease in glucose levels, reductions in MDA and NADPH activities, and an enhancement of SOD and CAT activities. Similarly, serum activities of antioxidant enzymes such as SOD, CAT, glutathione peroxidase (GPx), and glutathione S-transferase (GST) significantly improved following oral quercetin treatment in the diabetic model, demonstrating its antioxidative properties (29).

In the present study, which focuses on a fructose-induced MetS model, we observed trends consistent with literature. Quercetin modulated oxidative stress markers, including MDA, advanced oxidation protein products (AOPP), and TOS within liver tissue. However, distinct differences in metabolic pathways between diabetes and fructose-induced MetS models may explain variations in quercetin's effects on parameters such as NO and TAS. The oxidative burden and response lag observed in our model suggest that disease-specific factors, quercetin dosage, and duration critically influence its antioxidant efficacy. These findings collectively underscore the importance of context-specific evaluations of quercetin's therapeutic potential, bridging insights from diverse metabolic conditions.

These results collectively suggest that quercetin may exert protective effects on the oxidative stress associated with diabetes mellitus, potentially by modulating antioxidant enzyme activities. However, the precise mechanisms involved in quercetin's antioxidant actions and its impact on specific pathways warrant further investigation for a comprehensive understanding of its therapeutic potential in diabetes-related oxidative imbalance.

Flavonoids are known for their diverse biological activities, including the modulation of oxidative stress, inflammation, and apoptosis, which are critical in various metabolic and disease models. Previous research on different flavonoids has demonstrated their ability to reduce oxidative stress and apoptosis in animal models of diabetes induced by distinct mechanisms. For example, one study investigated a flavonoid's efficacy in alleviating pancreatic β-cell apoptosis by modulating pro-apoptotic pathways and oxidative stress markers in a rat model of diabetes. Although the model and specific pathways differ from our study, these findings highlight the conserved mechanisms through which flavonoids, including quercetin, exert their therapeutic effects. These parallel underscores quercetin's potential as a promising candidate for addressing oxidative stress-related metabolic disorders (34).

Furthermore, when comparing the tissue OSI values among different groups, no statistically significant difference was observed ($p=0.685$) in our study. These findings suggest that, at the administered dose, quercetin did not exert a dominant oxidant or antioxidant effect. The implications of these results underscore the need for further exploration into the nuanced effects of quercetin at different doses, durations, and under various experimental conditions to fully comprehend its potential benefits in oxidative stress management in the context of diabetes.

The concept that every organism begins its life cycle with a specific energy reserve capacity, which is utilized in proportion to metabolic speed, aligns with how metabolic processes like fructose metabolism can influence oxidative stress (1,35). Increased metabolic activity, such as the enhanced activity of the polyol pathway and mitochondrial dysfunction during fructose metabolism, leads to the generation of reactive oxygen species (ROS). These ROS interact with organic molecules, contributing to oxidative damage that may affect lifespan (34). Markers such as MDA and AOPP directly reflect lipid and protein

oxidation resulting from ROS, while TAS and NO are influenced by both enzymatic and non-enzymatic antioxidant systems. The response of these antioxidant systems may take longer to exhibit significant changes, suggesting a delayed counteraction to oxidative damage. This delay highlights how the rate of oxidative stress and the body's ability to manage it may influence the balance between energy consumption, oxidative damage, and overall health outcomes.

In our study, a comprehensive assessment of oxidative stress markers revealed that fructose administration led to a statistically significant increase in MDA (0.731 ± 0.05, $p = 0.004$), AOPP (0.81 ± 0.18, $p = 0.025$), and TOS (0.53 ± 0.01, $p = 0.037$) levels. However, the increase in NO (0.082 ± 0.04) and TAS (26.46 ± 4.4) levels did not reach statistical significance ($p>0.05$). This finding highlights the complexity of oxidative stress mechanisms, which may not solely depend on these markers.

Previous studies have demonstrated that the dose and duration of fructose exposure significantly influence oxidative stress parameters. For instance, a study reported that prolonged exposure to high doses of fructose led to significant alterations in both pro-oxidant and antioxidant markers (7). In our study, the moderate dose and relatively short duration of fructose administration may have limited its impact on NO and TAS levels.

The observed discrepancies in oxidative stress responses may also reflect tissue-specific variations in antioxidant defense systems. For example, TAS reflects the cumulative antioxidant capacity of non-enzymatic and enzymatic components, which can vary depending on the metabolic state and compensatory mechanisms activated during oxidative stress. Similarly, NO production may be influenced by additional regulatory pathways, including nitric oxide synthase activity and substrate availability. These findings highlight the nuanced interplay between oxidative damage and antioxidant defense in fructose-induced oxidative stress.

These findings underscore the importance of considering both dose and duration in studies examining fructose-induced oxidative stress. While our results clearly demonstrate oxidative damage as evidenced by increased MDA, AOPP, and TOS, the nuanced responses of NO and TAS highlight the complexity of the oxidative stress-antioxidant defense balance in liver tissue. Future studies employing varying doses and durations of fructose exposure could further elucidate these dynamics and provide a deeper understanding of the mechanisms underlying these responses.

Interestingly, when quercetin was co-administered with fructose, we observed notable changes in oxidative stress markers. The levels of MDA, TAS, and TOS showed no significant difference compared to the fructose-only group ($p>0.05$), suggesting that quercetin may have a balancing effect on oxidative stress. This observation is in line with previous studies suggesting that quercetin can modulate oxidative stress, although its effect may be dose-dependent or influenced by the duration of treatment. In contrast, both AOPP (0.33 ± 0.14, $p=0.004$) and NO (0.052 ± 0.02) levels showed a marked decrease, with the reduction in AOPP reaching statistical significance ($p=0.004$). This indicates that quercetin may effectively counteract fructose-induced oxidative damage, particularly by inhibiting protein oxidation pathways. These findings support the notion that quercetin, through its antioxidant properties, may attenuate the oxidative stress associated with metabolic dysfunction induced by fructose.

Furthermore, the administration of quercetin alone did not result in significant alterations in the evaluated parameters, a finding consistent with current literature, which similarly shows limited studies examining liver tissue markers beyond NO. The subtle effects of quercetin on oxidative stress indicators underscore the need for further research to clarify its potential hepatoprotective properties and its complex role in sustaining redox balance. Such insights could advance our understanding of quercetin's nuanced impact on liver health and its broader therapeutic applications.

CONCLUSION

In our study, the induction of the MetS model was successfully achieved through a 10-week administration of 20% fructose in the drinking water of experimental animals. This resulted in the manifestation of hypertension, hypertriglyceridemia, increased insulin levels, and insulin resistance, meeting the criteria indicative of MetS. Subsequently, the antioxidant potential of quercetin at this specific dose was meticulously investigated in the liver tissue.

To ascertain obesity in the MetS rat model, the Lee index was calculated. Quercetin was administered both independently and with fructose to explore its therapeutic effect on MDA, NO, TAS, and TOS levels. Our study contributes to understanding quercetin's impact on metabolic parameters in MetS, particularly its role in mitigating oxidative stress.

The MetS model was successfully established through fructose administration, leading to obesity, hypertension, hypertriglyceridemia, hyperglycemia, and insulin resistance. Quercetin was effective in ameliorating hypertension and insulin resistance but had limited effects on obesity, hyperglycemia, and hypertriglyceridemia.

Analysis of oxidative stress markers revealed a significant increase in MDA, AOPP, and TOS levels in fructose groups compared to controls. However, the increase in NO and TAS levels was not significant. No differences were observed between the control and quercetin groups in any parameter.

In conclusion, our study suggests that a 10-week administration of quercetin may be beneficial, particularly in reducing protein oxidation in the fructose-mediated MetS model. Future studies with larger sample sizes and diverse methodologies could provide broader insights into these findings.

In future investigations, it is imperative for quercetin studies to delve into elucidating the intricate mechanisms underlying obesity and insulin resistance within Metabolic Syndrome models. This exploration should encompass diverse doses and durations of quercetin administration, aiming to unravel potential synergistic effects when combined with other antioxidant drugs. We advocate for comprehensive research projects that thoroughly investigate the molecular targets and mechanisms of action of quercetin, utilizing advanced methodologies such as transcriptomic, proteomic, and metabolomic analyses. Such multifaceted approaches will provide deeper insights

into quercetin's therapeutic potential and elucidate its role in modulating cellular pathways. Such endeavors hold the promise of providing profound insights into the pathogenesis of MetS and offering innovative avenues for its treatment.

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