The Potential Ameliorative Effects of the Noisome Encapsulated Rutin or Quercetin Nano-Particles in HFD-Induced Metabolic Disorders in Male Rats

Khalifa El-Dawy[1*], Saydat Saad[1], Rasha Yahia[1], and Tarek Khamis[2*]

1Biochemistry Department, Faculty of Veterinary Medicine, Zagazig University, 44519 Zagazig, Egypt

2Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, 44519 Zagazig, Egypt

E-mail: r80anas@gmail.com, T.khamis@vet.zu.edu.eg

Conflict of interest: None declared

Funding: No funding sources

Abstract

Background: Rutin and quercetin, which are flavonoids found in many fruits and vegetables, have attracted interest due to their ability to improve metabolic syndrome and its related testicular dysfunction. Gaining insight into the mechanisms by which they operate and investigating innovative delivery technologies, such as nanotechnology, may present effective approaches for addressing these health issues. Objective: The purpose of this study was to examine the effectiveness of rutin and quercetin-loaded niosomes in reducing testicular dysfunction related to metabolic syndrome in a rat model. Methodology: The ethanol injection method was employed to produce niosomes containing rutin and quercetin. These niosomes were then evaluated for several parameters. A total of ninety adult male albino rats were subjected to metabolic syndrome induction by a high-fructose diet for a duration of 10 weeks. Afterward, the rats were separated into six distinct groups. Various treatment protocols were delivered, including the use of niosomes filled with rutin and quercetin. The evaluation focused on metabolic indicators, inflammatory mediators, and oxidative stress markers. Results: The results showed that the niosomes containing rutin and quercetin had suitable particle size, polydispersity index, and zeta potential. Additionally, they demonstrated good levels of encapsulation and drug loading efficiency. The administration of rutin and quercetin-loaded niosomes resulted in a substantial enhancement in insulin resistance, lipid profile, adipokine levels, inflammatory mediators, and oxidative stress indicators when compared to the control and obesity groups. In conclusion, the results emphasize the effectiveness of niosomal delivery methods in specifically targeting various pathophysiological pathways associated with obesity and metabolic syndrome. This opens new possibilities for innovative therapeutic approaches in the treatment of this condition.

Keywords: Rutin, Quercetin, Metabolic syndrome, Noisome

TobRegul Sci. ™ 2023; 9(1): 7097 - 7112

DOI: doi.org/10.18001 /TRS.9.1.502

1. Introduction

Metabolic syndrome, a collection of interrelated risk factors such as obesity, hypertension, insulin resistance, and dyslipidemia, presents a substantial danger to worldwide health. The occurrence of this phenomenon has significantly increased in recent years, closely mirroring the upward trend in inactive lifestyles and bad eating patterns [1]. Rutin and quercetin, which are well-known flavonoids, have a wide range of biological effects such as antioxidant, anti-inflammatory, and anti-diabetic qualities. These substances have shown effectiveness in reducing various aspects of metabolic syndrome by influencing important pathways related to glucose and lipid metabolism, oxidative stress, and inflammation [4]. Niosomes, which are non-ionic surfactant vesicles, offer a promising method for delivering drugs and have the ability to address the difficulties associated with administering bioactive chemicals like rutin and quercetin. The nanostructured lipid carriers are comprised of self-assembled bilayers made up of non-ionic surfactants and cholesterol. They possess benefits such as biocompatibility, biodegradability, and adjustable physicochemical features. Niosomes have the ability to enclose hydrophilic pharmaceuticals in their aqueous core and hydrophobic drugs in their lipid bilayers. This property improves the solubility, stability, and bioavailability of the drugs. In addition, the adaptable characteristics of niosomes make it possible to modify their surface with targeting ligands or stimuli-responsive polymers. This enables the precise delivery of drugs to specified sites and regulated release [8]. Adiponectin, a hormone mostly released by fat cells, demonstrates anti-inflammatory effects and enhances the body's response to insulin. Individuals with obesity and metabolic syndrome often exhibit reduced levels of adiponectin, which indicates malfunction in adipose tissue and chronic inflammation [9]. Leptin, an additional adipokine, controls energy equilibrium and hunger by affecting the central nervous system. Increased levels of leptin, which are a feature of obesity, indicate a resistance to leptin and a disruption in the balance of energy regulation. Leptin resistance interferes with the neuroendocrine control of the HPG axis, resulting in hypogonadism and reduced testicular function [10]. Ghrelin, a peptide hormone predominantly synthesized in the stomach, controls hunger and the utilization of energy. The dysregulation of ghrelin signaling, observed in individuals with metabolic syndrome, can disturb the regulation of hunger, facilitate weight gain, and worsen insulin resistance [11].

The objective of our study is to assess the effectiveness of rutin and quercetin loaded niosomes in modulating the hepatic affection associated with metabolic syndrome in rats. Also, to improve the effectiveness and availability of these medicines by enclosing them in niosomal carriers. This will allow us to specifically target the several variables that contribute to metabolic syndrome.

2 Materials and methods

2.1 Formulation and Characterization of rutin and quercetin-loaded Niosomes

Initially, niosomes containing rutin and quercetin were synthesized using the ethanol injection method. The produced systems underwent examination using several parameters including particle size, polydispersity index, zeta potential, encapsulation efficiency, drug loading efficiency, scanning electron microscopy, in vitro release research, and stability study [17].

2.2 Animals and experimental design

A total of 90 adult male albino rats were obtained from the animal facility at the Faculty of Veterinary Medicine-Zagazig University. The rats were between 8 and 10 weeks old and had an average weight of 180 to 200 grams. The rats were provided with standard care in accordance with laboratory animal protocols, and the study was approved by the Zagazig University institutional committee for animal care and use (approval number: ZU-IACUC/3/F/76/2022). Following a one-week period of acclimatization, rats were divided into 6 equal groups at random.

2.2.1 Induction of Metabolic Syndrome

The rats were administered a high fructose diet for a duration of 10 weeks in order to produce metabolic syndrome. The high fructose diet consists of 60% of the total calories derived from fructose. It is created by adding 60% weight/weight fructose to a conventional mouse diet [18]. Following a 10-week period of administering a high-fat diet, the presence of fructose-induced metabolic syndrome was confirmed by observing changes in body weight and a notable increase in fasting serum glucose, insulin, and triglycerides. Rats were then utilized for the post-treatment duration of 4 weeks.

Group I, the control group consisted of rats that were provided with a regular chow diet for the whole duration of the trial. Instead of using supplements, the experimental groups were given 0.9% saline orally to ensure equal treatment.

Group II: designated as the obesity group, consisted of rats that were given a high-fructose diet and administered a saline solution, similar to the control group, for the whole duration of the experiment.

Group III: rats were administered a high-fructose diet and administered a QU-NIO (0.3 mg/kg b.wt/day) by intragastric intubation before and after metabolic syndrome induction and act as protective group (pro-QU).

Group IV: rats were administered a high-fructose diet and administered a RU-NIO (0.3 mg/kg b.wt/day) by intragastric intubation before and after metabolic syndrome induction and act as protective group (pro-RU).

Group V: Following the confirmation of metabolic syndrome, rats were administered a high-fructose diet and administered a QU-NIO (0.3 mg/kg b.wt/day) by intragastric intubation. This therapy began in week 11 of the experimental period, after verifying the presence of metabolic syndrome and act as treatment group (TT-QU).

Group VI: Following the confirmation of metabolic syndrome, rats were administered a high-fructose diet and administered a RU-NIO (0.3 mg/kg b.wt/day) by intragastric intubation. This therapy began in week 11 of the experimental period, after verifying the presence of metabolic syndrome and act as treatment group (TT-RU).

2.3 Hematological specimen collection

In each group after 4 weeks of intervention numbers of rats were euthanizedand blood sample were obtained to confirm some biochemical test and after 8-week intervention period and a 12-hour fasting period, the rats were euthanizedwith an intraperitoneal injection of sodium thiopental at a dosage of 60 mg/kg. Subsequently, blood samples were obtained via cardiac puncture, allowed to clot, and subsequently subjected to centrifugation at a speed of 3,000 round per minute for a duration of 15 minutes. The serum samples were stored at a temperature of -20 °C until they were utilized for biochemical analysis after 4 weeks and 8 weeks.

2.4 Specimen of tissue

The hepatic and adipose tissues of the rats were collected and promptly placed in a liquid nitrogen container, wrapped with aluminum foil, and stored at a temperature of -80°C for other biochemical analysis.

2.5 Evaluation of biochemical parameters

An automated biochemistry analyzer, namely the Cobas® 6,000 analyzer from Roche Diagnostics Ltd. in Switzerland, was used to perform enzymatic colorimetry. This method was employed to evaluate various parameters including fasting blood glucose (FBG) using glucose enzymatic (GOD-PAP) kits, and serum lipid profile. The HOMA-IR (homeostatic model assessment of insulin resistance) was determined using the following formula: HOMA-IR = fasting serum glucose (mg/dL) multiplied by fasting serum insulin (μIU/mL), divided by 405 [19].

2.6 Evaluation of oxidative stress markers.

The hepatic levels of MDA, SOD, GPX, and CAT, in the hepatic and testicular homogenate using a commercially available kit (Biodiagnostic, Giza, Egypt) according to the manufacturer's instructions. The measurements were done using a spectrophotometer (Spectronic 3,000 Array, Germany).

2.7 Statistical analysis

The data were presented as the mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism®, Version 9.2, a software developed by GraphPad Software Inc. in San Diego, California, United States. The data exhibited a normal distribution and were subjected to analysis using an ANOVA test to compare means, followed by Tukey-Kramer multiple comparisons tests. A p-value less than 0.05 was deemed statistically significant.

3. Results

3.1 Effect of QU-NIO and RU-NIO on the glycemic parameters of the HFD indued rat model.

The results showed a significant ($p < 0.0001$) increased in insulin, FBS, and HOMA-IR levels in treated and protective groups of QU-NIO and RU-NIO than control group, but their level was decreased in comparison with obese group when used QU-NIO and RU-NIO in protective and treated groups after 4, and 8 weeks. The best results were detected in QU-NIO treated and protective groups than RU-NIO groups after 8 weeks (Figure 1).

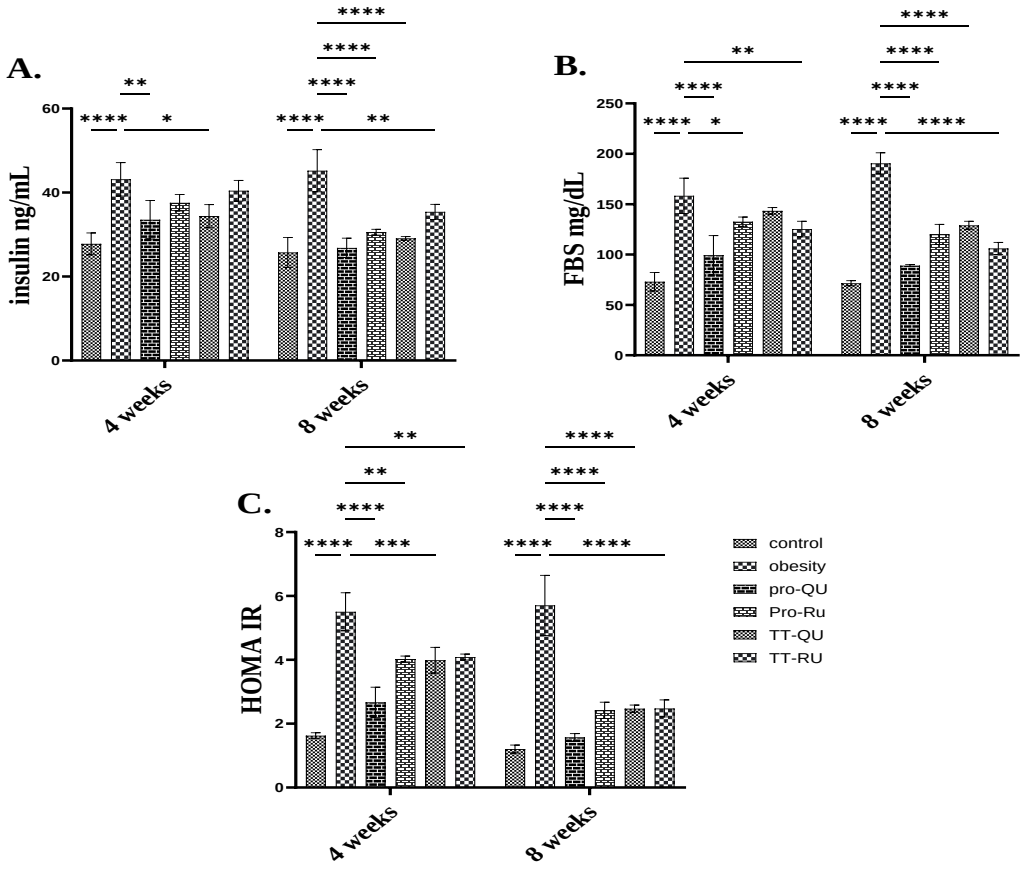

Figure 1.
Effect of QU-NIO and RU-NIO on insulin (ng/mL), FBS (mg/mL), and HOMA-IR.

Data are expressed as means ± SEM. * p < 0.05=mild significant, ** p < 0.01=moderate significant, *** p < 0.001, *** p < 0.0001, indicate highly significant difference.

3.2 Effect of QU-NIO and RU-NIO on the lipid profile of the HFD indued rat model.

The findings demonstrated a statistically significant (p < 0.0001) rise in TAG, Total cholesterol, and LDL levels in the treated and protective groups of QU-NIO and RU-NIO compared to the control group. However, these levels were lower when QU-NIO and RU-NIO were administered in the protective and treated groups after 4 and 8 weeks, in comparison to the obese group. except for HDL levels were increased in prophylactic and treatment groups in comparison with obese groups. After 8 weeks, the QU-NIO treated and protected groups showed superior results compared to the RU-NIO groups (Figure 2).

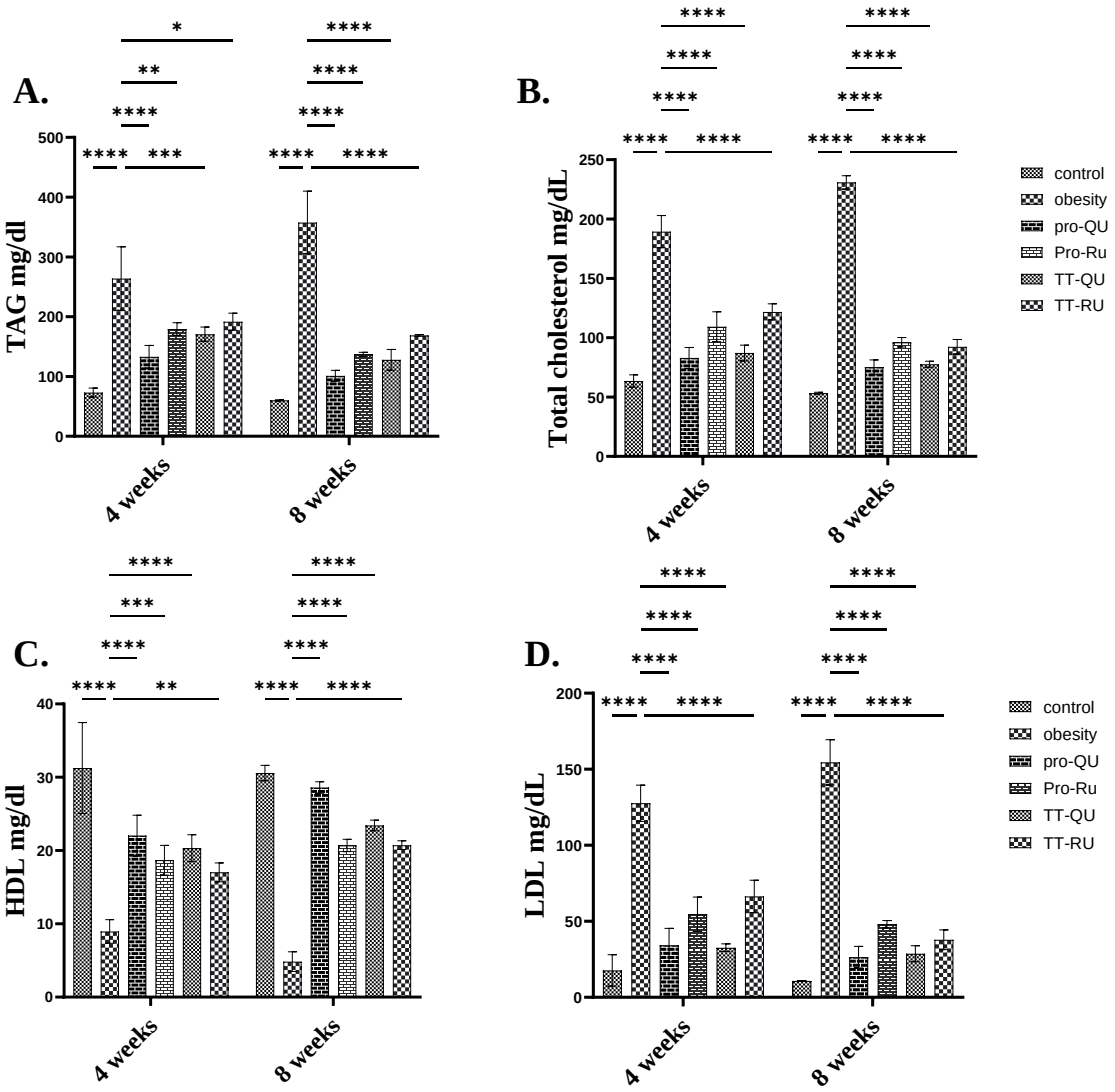

Figure 2. Effect of QU-NIO and RU-NIO on lipid profile. Data are expressed as means ± SEM. * p < 0.05=mild significant, ** p < 0.01=moderate significant, *** p < 0.001, *** p < 0.0001, indicate highly significant difference.

3.3. Effect of QU-NIO and RU-NIO on the oxidative stress markers of the HFD indued rat model.

Our data revealed a significant (p < 0.0001) increased in Hepatic MDA (nmol/mg), levels in treated and protective groups of QU-NIO and RU-NIO than control group, but their levels were decreased in comparison with obese group when used QU-NIO and RU-NIO in protective and treated groups. Hepatic SOD (U/mg), and Hepatic GPX (ng/mg) levels were decreased in comparison with control group. The best results were detected in QU-NIO treated and protective groups than RU-NIO groups after 8 weeks' treatment (Figure 3).

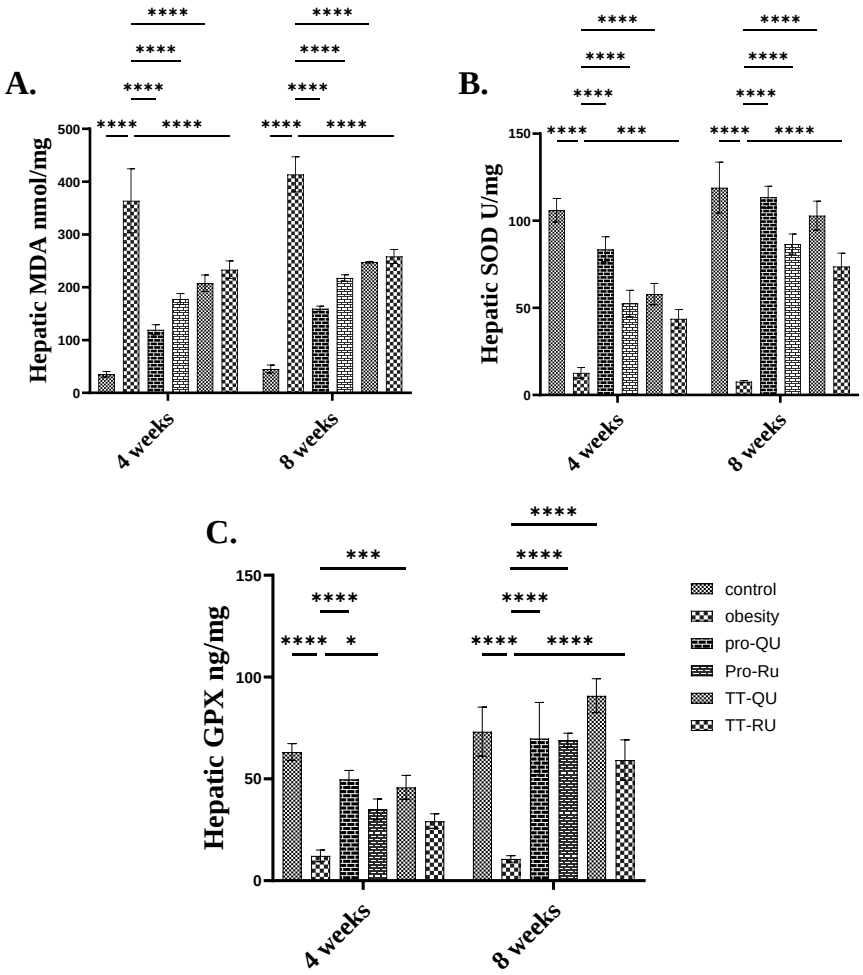

Figure 3. Effect of QU-NIO and RU-NIO on hepatic oxidative stress markers. Data are expressed as means ± SEM. * p < 0.05=mild significant, ** p < 0.01=moderate significant, *** p < 0.001, *** p < 0.0001, indicate highly significant difference.

4. Discussion

MetS, or metabolic syndrome, is a global health issue that can result in the emergence of cardiovascular and cerebrovascular diseases, which are presently among the leading causes of mortality, alongside metabolic disorders. Consequently, significant measures are taken to decrease the occurrence and progression of MetS using both pharmaceutical and non-pharmacological means [21]. Our study focused on examining the impact of the natural compound rutin and the quercetin loaded niosome on a condition similar to MetS that was caused by a High-Fructose Diet in rats. The results of our study verified our hypothesis that the administration of rutin and/or the quercetin loaded niosome can enhance metabolic indicators, and hepatic oxidative stress markers.

Encapsulating phytochemicals into polymeric nanoparticles or conjugating them to polymers improves their bioavailability and increases their sustained and regulated release rate, as well as enhancing their water solubility and stability. Polymeric nanoparticles possess unique surface chemistry that enables them to adsorb, entrap, or covalently attach medicinal phytochemicals, resulting in a significant improvement in drug delivery [22].

The characterisation of drug-loaded nanocarriers is of great importance in order to obtain a highly effective drug delivery vehicle. This characterization process allows for the thorough examination of the physicochemical properties of the therapeutic carrier system, which in turn helps to closely monitor its behavior at the targeted site of action. The size distribution in this scenario is a significant factor that directly affects the drug release profile, physical stability, cellular absorption, in vivo bioavailability, and bio-distribution of the drug-loaded nanocargo [23].

Another study showed that rats who were given a high-fat diet for 12 weeks experienced obesity, which was marked by an elevation in body weight, body length, total cholesterol, abdominal circumference, body mass index, and Lee index. Previous research has demonstrated that variations in weight and blood lipid profile can be utilized to determine the effective achievement of obesity in animal model 50. In addition, Gaur et al. [27] reported that supplementing rats with an unsaturated high-fat diet leads to an augmentation in weight gain, overall body fat, and specific fat deposits such as retroperitoneal, visceral, and epididymal fat pads. The study conducted by Wang et al. [28] revealed that animals fed a high-fat diet for 10 weeks had a weight that was more than 20% higher than the control animals. Additionally, the Lee's index, waist circumference, and visceral fat mass were all significantly greater in the high-fat diet group compared to the control group. Therefore, the obesity in the animals fed a high-fat diet could be attributed to the increased presence of fatty substances resulting from the high-fat diet regimen [29].

The present study found that extended high-fat diet feeding resulted in a disruption of lipid metabolism, as evidenced by elevated levels of cholesterol, triglycerides, and low-density lipoprotein, together with decreased levels of high-density lipoprotein. This observation is well corroborated by the studies conducted by Noeman et al. [30], Kabir et al. [31], and Othman et al. [29].

Consuming a high-fat diet has been observed to induce oxidative stress, a condition that is evident in the majority of experimental models and individuals with clinical illnesses [32]. Currently, obese rats exhibited an increase in the amount of the lipid peroxidation product, MDA, and a decrease in TAC. Our findings align with earlier studies indicating that obese animals may experience reduced tissue antioxidant defenses as a result of prolonged intake of a high-fat diet [33]. The current work reveals that untreated obese rats experience oxidative stress due to an imbalance between free radical production and antioxidant level, leading to a decreased antioxidant defense system. Obese rats exhibit a compromised antioxidant defense mechanism, leading to lipid peroxidation damage. Ahmed and colleagues [24] discovered that mice fed a high-fat diet exhibited elevated levels of thiobarbituric acid reactive compounds, which are recognized as markers of lipid peroxidation. A significant contributor to the reduction in fluidity of the mitochondrial membrane and the promotion of ROS production is the consumption of a large number of calories. Consequently, an increase in ROS concentration might overwhelm the body's antioxidant defenses, resulting in oxidative stress.

Recent evidence has demonstrated that ROS interact with protein thiol molecules to produce various sulfur oxidations. These oxidations negatively affect the receptor signal of insulin and hinder the cellular uptake of triglycerides from the bloodstream. The latest evidence indicates that the administration of obese rats with free Quercetin, and Quercetin nanoparticles, resulted in a reduction in serum cholesterol, triglyceride, and low-density lipoprotein levels, while simultaneously increasing serum high-density lipoprotein levels [24]. The hypolipidemic benefits of Qu may mostly be attributed to its antioxidant qualities, as well as its indirect influence on cholesterol levels. Another possibility suggests that Qu plays a role in reducing the activity of HMG-CoA reductase, which in turn decreases cholesterol production in the liver [34]. In addition, there is increasing evidence that Qu and its glycoside work by modifying the absorption of cholesterol in the liver and the assembly and secretion of triglycerides. They also decrease phosphodiesterases in the liver and adipose tissue [34].

During the ongoing experiment, rats who were obese displayed elevated amounts of glucose and insulin in their bloodstream, together with a significant insulin resistance value. This finding is consistent with prior research that demonstrated rats on a high-fat diet exhibited elevated levels of glucose in their bloodstream. In addition, Ahmed et al. [24] reported that feeding rats with a high-fat diet for 8 weeks led to significantly elevated levels of fasting glucose and fasting insulin,

as well as a higher HOMA-IR, indicating an increase in insulin resistance compared to the control group. The observed phenomena can be attributed to the presence of lipopolysaccharide (LPS) in the cell wall of gram-negative bacteria. This LPS may enter the gut due to the permeability of the intestinal mucosa, which is caused by a high fat content in the diet.

Abundant research has demonstrated that nanoparticles loaded with Quercetin have greater anti-radical and antioxidative properties in comparison to free Quercetin. Encapsulation of Quercetin into PLGA-NPs enhanced its effectiveness as a lipid oxidation inhibitor and its antioxidative effects. Furthermore, the incorporation of Qu into aminoalkyl methacrylate co-polymers, specifically poly vinyl alcohol NPs (nanoparticles), resulted in an augmentation of the anti-radical and antioxidant properties of Qu [35]. Consistent with these investigations, our data demonstrated that the effectiveness of Qu NPs in rebalancing the oxidant/anti-oxidant ratio in obese rats was great. Ting et al. [36] have verified that administering Qu to obese rats reduces plasma MDA levels and enhances TAC. In addition, Al-Jameel and Abd El-Rahman [37] observed that administering Qu NPs to diabetic rats decreases the concentration of MDA in the blood and increases the activity of antioxidant enzymes.

Supplementing animal models with Quercetin resulted in decreased levels of glucose, insulin, triglycerides, and cholesterol. However, it also increased levels of serum adiponectin [38]. Wein et al. [39] found that quercetin had a direct effect on high fat-fed rats, regardless of their body fat level. Quercetin increases insulin sensitivity and the HOMA index [39].

Rutin, also known as vitamin P, is a compound that is abundant in various vegetables, fruits, and therapeutic foods such as asparagus and buckwheat. It offers numerous physiological health advantages [40]. The in vivo bioactivity of buckwheat supplement against T2D seems to be correlated with the ingestion of rutin, which contributes to its hypoglycemic activity. Furthermore, the use of 0.1% rutin yielded the most optimal result [40].

5. Conclusion

Overall, this work highlights the potential therapeutic effectiveness of rutin and quercetin-loaded niosomes in improving testicular dysfunction related to metabolic syndrome. The thorough assessment of biochemical indicators, oxidative stress markers, and metabolic indices reveals the diverse advantages of niosomal delivery systems in targeting crucial pathways involved in metabolic syndrome-associated disorders. These encouraging findings justify the need for more research and clinical trials to confirm the effectiveness and safety of rutin and quercetin-loaded niosomes as a potential treatment for metabolic syndrome disorders.

6. Limitation of the current study

Additional research is needed to clarify the prolonged consequences and possible negative responses of rutin and quercetin-loaded niosomes in various animal models and human subjects who have metabolic syndrome. It is crucial to investigate the most effective dosage, frequency, and duration of rutin and quercetin-loaded niosome therapy in order to achieve the highest therapeutic effectiveness while minimizing any potential adverse effects. This research is necessary for the successful application of this therapy in clinical settings. In order to apply the encouraging preclinical results of rutin and quercetin-loaded niosomes to real-world medical practice, it is necessary to conduct thorough clinical trials that assess their safety, effectiveness, and long-term advantages in enhancing testicular health for individuals with metabolic syndrome.

Conflict of interest: the authors declare that there is no competing interest. All authors read, revise, and approve the current work for possible publication.

Funding: the current work did not receive funding neither internal nor external.

Author contribution: all the author contributed equally to the current work. Prof. Dr. Khalifa El-Dawy supervised the current work.

References:

1. Lemieux, I., & Després, J. P. (2020). Metabolic syndrome: past, present and future. Nutrients, 12(11), 3501.
2. Bovolini, A., Garcia, J., Andrade, M. A., & Duarte, J. A. (2021). Metabolic syndrome pathophysiology and predisposing factors. International journal of sports medicine, 42(03), 199-214.
3. Fahed, G., Aoun, L., Bou Zerdan, M., Allam, S., Bou Zerdan, M., Bouferraa, Y., & Assi, H. I. (2022). Metabolic syndrome: updates on pathophysiology and management in 2021. International journal of molecular sciences, 23(2), 786.
4. da Silva, A. B., Coelho, P. L. C., das Neves Oliveira, M., Oliveira, J. L., Amparo, J. A. O., da Silva, K. C., ... & Costa, S. L. (2020). The flavonoid rutin and its aglycone quercetin modulate the microglia inflammatory profile improving antiglioma activity. Brain, behavior, and immunity, 85, 170-185.
5. Wani, T. A., Bakheit, A. H., Zargar, S., Alanazi, Z. S., & Al-Majed, A. A. (2021). Influence of antioxidant flavonoids quercetin and rutin on the in-vitro binding of neratinib to human serum albumin. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 246, 118977.
6. Pinheiro, R. G., Pinheiro, M., & Neves, A. R. (2021). Nanotechnology innovations to enhance the therapeutic efficacy of quercetin. Nanomaterials, 11(10), 2658.

7. Negahdari, R., Bohlouli, S., Sharifi, S., Maleki Dizaj, S., Rahbar Saadat, Y., Khezri, K., ... & Raeesi, S. (2021). Therapeutic benefits of rutin and its nanoformulations. Phytotherapy Research, 35(4), 1719-1738.

8. Bhardwaj, P., Tripathi, P., Gupta, R., & Pandey, S. (2020). Niosomes: A review on niosomal research in the last decade. Journal of Drug Delivery Science and Technology, 56, 101581.

9. Lee, S. K., Yoon, D. W., Kim, J., Lee, S. W., Kim, J. Y., Jee, S. H., ... & Shin, C. (2018). Association of adiponectin, ghrelin, and leptin with metabolic syndrome and its metabolic components in Sasang constitutional type. European Journal of Integrative Medicine, 22, 16-21.

10. Ghadge, A. A., & Khaire, A. A. (2019). Leptin as a predictive marker for metabolic syndrome. Cytokine, 121, 154735.

11. Kim, S., Nam, Y., Shin, S. J., Park, Y. H., Jeon, S. G., Kim, J. I., ... & Moon, M. (2020). The potential roles of ghrelin in metabolic syndrome and secondary symptoms of Alzheimer's disease. Frontiers in Neuroscience, 14, 583097.

12. Monserrat-Mesquida, M., Quetglas-Llabrés, M., Capó, X., Bouzas, C., Mateos, D., Pons, A., ... & Sureda, A. (2020). Metabolic syndrome is associated with oxidative stress and proinflammatory state. Antioxidants, 9(3), 236.

13. Carmo de Carvalho e Martins, M. D., Martins, da Silva Santos Oliveira, A. S., da Silva, L. A. A., Primo, M. G. S., & de Carvalho Lira, V. B. (2022). Biological indicators of oxidative stress [malondialdehyde, catalase, glutathione peroxidase, and superoxide dismutase] and their application in nutrition. In Biomarkers in Nutrition (pp. 1-25). Cham: Springer International Publishing.

14. Iwasa, T., Matsuzaki, T., Yano, K., Mayila, Y., & Irahara, M. (2018). The roles of kisspeptin and gonadotropin inhibitory hormone in stress-induced reproductive disorders. Endocrine Journal, 65(2), 133-140.

15. Khan, S. H., & Chaudhry, N. (2021). Beyond GnRH, LH and FSH: The role of kisspeptin on hypothalalmic-pituitary gonadal (HPG) axis pathology and diagnostic consideration. J. Pak. Med. Assoc, 71, 1862-1869.

16. Zhao, L., & Pang, A. (2020). Effects of Metabolic syndrome on semen quality and circulating sex hormones: a systematic review and meta-analysis. Frontiers in Endocrinology, 11, 544553.

17. Mostafa, M., Alaaeldin, E., Aly, U. F., & Sarhan, H. A. (2018). Optimization and characterization of thymoquinone-loaded liposomes with enhanced topical anti-inflammatory activity. Aaps Pharmscitech, 19, 3490-3500.

18. Maithilikarpagaselvi, N., Sridhar, M. G., Swaminathan, R. P., Sripradha, R., & Badhe, B. (2016). Curcumin inhibits hyperlipidemia and hepatic fat accumulation in high-fructose-fed male Wistar rats. Pharmaceutical Biology, 54(12), 2857-2863.

19. Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. diabetologia, 28, 412-419.

20. Dawson, B., & Trapp, R. G. (2004). Basic & clinical biostatistics. In Basic & Clinical Biostatistics (pp. 438-438).

21. Greenfield, D. M., & Snowden, J. A. (2019). Cardiovascular diseases and metabolic syndrome. The EBMT handbook: Hematopoietic stem cell transplantation and cellular therapies, 415-420.

22. Goktas, Z., Zu, Y., Abbasi, M., Galyean, S., Wu, D., Fan, Z., & Wang, S. (2020). Recent advances in nanoencapsulation of phytochemicals to combat obesity and its comorbidities. Journal of agricultural and food chemistry, 68(31), 8119-8131.

23. Mu, H., & Holm, R. (2018). Solid lipid nanocarriers in drug delivery: characterization and design. Expert opinion on drug delivery, 15(8), 771-785.

24. Ahmed, H. H., Kotob, S. E., Abd-Rabou, A. A., Aglan, H. A., Elmegeed, G. A., & Mohawed, O. A. (2021). Pre-clinical evidence for the anti-obesity potential of quercetin and curcumin loaded chitosan/PEG blended PLGA nanoparticles. Biomedical and Pharmacology Journal, 14(4), 1731-1759.

25. Abd El-Fattah, A. I., Fathy, M. M., Ali, Z. Y., El-Garawany, A. E. R. A., & Mohamed, E. K. (2017). Enhanced therapeutic benefit of quercetin-loaded phytosome nanoparticles in ovariectomized rats. Chemico-biological interactions, 271, 30-38.

26. Bais, S., Singh, G. S., & Sharma, R. (2014). Antiobesity and hypolipidemic activity of Moringa oleifera leaves against high fat diet-induced obesity in rats. Advances in Biology, 2014.

27. Gaur, A., Pal, G. K., Ananthanarayanan, P. H., & Pal, P. (2014). Role of Ventromedial hypothalamus in high fat diet induced obesity in male rats: association with lipid profile, thyroid profile and insulin resistance. Annals of Neurosciences, 21(3), 104.

28. Wang, C., Ha, X., Li, W., Xu, P., Gu, Y., Wang, T., ... & Zhang, J. (2017). Correlation of TLR4 and KLF7 in inflammation induced by obesity. Inflammation, 40, 42-51.

29. Othman, Z. A., Wan Ghazali, W. S., Noordin, L., Mohd. Yusof, N. A., & Mohamed, M. (2019). Phenolic compounds and the anti-atherogenic effect of bee bread in high-fat diet-induced obese rats. Antioxidants, 9(1), 33.

30. Noeman, S. A., Hamooda, H. E., & Baalash, A. A. (2011). Biochemical study of oxidative stress markers in the liver, kidney and heart of high fat diet induced obesity in rats. Diabetology & metabolic syndrome, 3, 1-8.

31. Kabir, F., Nahar, K., Rahman, M. M., Mamun, F., Lasker, S., Khan, F., ... & Alam, M. A. (2021). Etoricoxib treatment prevented body weight gain and ameliorated oxidative stress in the liver of high-fat diet–fed rats. Naunyn-Schmiedeberg's Archives of Pharmacology, 394, 33-47.

32. Mohammadi, A. A., Jazayeri, S., Khosravi-Darani, K., Solati, Z., Mohammadpour, N., Asemi, Z., ... & Eghtesadi, S. (2015). Effects of probiotics on biomarkers of oxidative stress and inflammatory factors in petrochemical workers: a randomized, double-blind, placebo-controlled trial. International journal of preventive medicine, 6(1), 82.

33. Ulla, A., Alam, M. A., Sikder, B., Sumi, F. A., Rahman, M. M., Habib, Z. F., ... & Reza, H. M. (2017). Supplementation of Syzygium cumini seed powder prevented obesity, glucose intolerance, hyperlipidemia and oxidative stress in high carbohydrate high fat diet induced obese rats. BMC complementary and alternative medicine, 17, 1-13.

34. Salama, Y. A., Hassan, H. M., El-Gayar, A. M., & Abdel-Rahman, N. (2024). Combined quercetin and simvastatin attenuate hepatic fibrosis in rats by modulating SphK1/NLRP3 pathways. Life Sciences, 337, 122349.

35. Wu, T. H., Yen, F. L., Lin, L. T., Tsai, T. R., Lin, C. C., & Cham, T. M. (2008). Preparation, physicochemical characterization, and antioxidant effects of quercetin nanoparticles. International journal of pharmaceutics, 346(1-2), 160-168.

36. Ting, Y., Chang, W. T., Shiau, D. K., Chou, P. H., Wu, M. F., & Hsu, C. L. (2018). Antiobesity efficacy of quercetin-rich supplement on diet-induced obese rats: effects on body composition, serum lipid profile, and gene expression. Journal of agricultural and food chemistry, 66(1), 70-80.

37. Al-Jameel, S. S., & Abd El-Rahman, S. N. (2017). Effect of quercetin nanoparticles on the kidney of the streptozotocin-induced diabetes in male rats: A histological study and serum biochemical alterations. African Journal of Biotechnology, 16(39), 1944-1952.

38. Kobori, M., Masumoto, S., Akimoto, Y., & Oike, H. (2011). Chronic dietary intake of quercetin alleviates hepatic fat accumulation associated with consumption of a Western-style diet in C57/BL6J mice. Molecular nutrition & food research, 55(4), 530-540.

39. Wein, S., Behm, N., Petersen, R. K., Kristiansen, K., & Wolffram, S. (2010). Quercetin enhances adiponectin secretion by a PPAR-γ independent mechanism. European Journal of Pharmaceutical Sciences, 41(1), 16-22.

40. Patel, K., & Patel, D. K. (2019). The beneficial role of rutin, a naturally occurring flavonoid in health promotion and disease prevention: A systematic review and update. Bioactive food as dietary interventions for arthritis and related inflammatory diseases, 457-479.

41. Zhao, S., Kusminski, C. M., & Scherer, P. E. (2021). Adiponectin, leptin and cardiovascular disorders. Circulation research, 128(1), 136-149.

42. Liu, J., Cao, J., Li, Y., & Guo, F. (2023). Beneficial flavonoid in foods and anti-obesity effect. Food Reviews International, 39(1), 560-600.

43. Lee, L. C., Hou, Y. C., Hsieh, Y. Y., Chen, Y. H., Shen, Y. C., Lee, I. J., ... & Liu, H. K. (2021). Dietary supplementation of rutin and rutin-rich buckwheat elevates endogenous glucagon-like peptide 1 levels to facilitate glycemic control in type 2 diabetic mice. Journal of Functional Foods, 85, 104653.

44. Fathy, M. A., Alsemeh, A. E., Habib, M. A., Abdel-Nour, H. M., Hendawy, D. M., Eltaweel, A. M., ... & Khamis, T. (2023). Liraglutide ameliorates diabetic-induced testicular dysfunction in male rats: role of GLP-1/Kiss1/GnRH and TGF-β/Smad signaling pathways. Frontiers in Pharmacology, 14, 1224985.

45. D'souza, A. M., Neumann, U. H., Glavas, M. M., & Kieffer, T. J. (2017). The glucoregulatory actions of leptin. Molecular metabolism, 6(9), 1052-1065.

46. Foudah, A. I., Salkini, M. A., Yusufoglu, H. S., Alkreathy, H. M., & Khan, R. A. (2023). Protective Effects of a Polyphenolic Phytochemical Quercetin against Oxidative Dysfunctions in Rats. Evidence-Based Complementary and Alternative Medicine, 2023.

47. Daim, N. E., & Al-Mayali, H. K. (2020). Effect of the rutin on azathioprine-induced toxicity in reproductive function male rats. Eurasian Journal of Biosciences, 14(2).

48. Kappel, V. D., Cazarolli, L. H., Pereira, D. F., Postal, B. G., Zamoner, A., Reginatto, F. H., & Silva, F. R. M. B. (2013). Involvement of GLUT-4 in the stimulatory effect of rutin

on glucose uptake in rat soleus muscle. Journal of Pharmacy and Pharmacology, 65(8), 1179-1186.