The Potential Implication of the Noisome Nanoparticle of the Rutin and Quercetin in Modulating the Obesity-Induced Testicular Dysfunction in Male Rats.

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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 obesity induced-testicular dysfunction in a rat model. Methods, A high-fructose diet was used to induce metabolic syndrome in 90 adult male albino rats over the course of ten weeks. The rats were then divided into six different groups. Numerous treatment plans were given, such as the application of rutin and quercetin-filled niosomes. The assessment was centred on markers of testicular oxidative stress and hypothalamic-pituitary gonadal axis. Results: Comparing the rutin and quercetin-loaded niosomes group to the control and obese groups, the results demonstrated a significant increase in oxidative stress markers and sex hormones. In addition, significant alterations in hypothalamic hormones linked to reproductive control were seen, indicating potential benefits for testicular function restoration. Conclusion, Testicular dysfunction associated with metabolic syndrome appears to be improving with the usage of niosomes enriched with rutin and quercetin. The outcomes highlight the efficiency of niosomal delivery techniques in precisely addressing several pathophysiological pathways linked to obesity-induced testicular failure. This creates fresh opportunities for cutting-edge therapeutic methods to address this illness.

Keywords: Rutin, Quercetin, Metabolic syndrome, Testicular dysfunction, Noisome, HPG axis

TobRegul Sci. ™ 2023; 9(1): 7082 - 7096

DOI: doi.org/10.18001 /TRS.9.1.501

1. Introduction

The metabolic syndrome, a group of linked risk factors that includes insulin resistance, obesity, hypertension, and dyslipidemia, poses a serious threat to global health. This problem has become much more common in recent years, closely tracking the rise in unhealthy eating habits and sedentary lifestyles [1]. Hormonal imbalances, oxidative stress, inflammation, and vascular dysfunction are some of the elements that define the intricate relationship between metabolic syndrome and testicular health. The hypothalamic-pituitary-gonadal (HPG) axis is disrupted by insulin resistance, a hallmark of metabolic syndrome, which lowers testosterone production and impairs spermatogenesis [2].

The development of damage in the testicular small blood vessels is a result of both dyslipidemia and hypertension. This hinders blood flow to the testicles and exacerbates the presence of hazardous oxidative chemicals in the testicular environment. Chronic inflammation is brought on by cytokines generated from adipose tissue, and this lowers sperm quality and Leydig cell function. The combined effects of these metabolic disturbances lead to testicular dysfunction, characterised by low testosterone levels, poor sperm production, and infertility [3]. Well-known flavonoids like quercetin and rutin have a variety of biological actions, including anti-inflammatory, anti-diabetic, and antioxidant properties. By impacting critical pathways linked to oxidative stress, inflammation, glucose and lipid metabolism, and other elements of metabolic syndrome, these compounds have demonstrated efficacy in mitigating its different manifestations [4]. Furthermore, current research suggests that these compounds improve testicular function. Studies have indicated that they can improve sperm quality, raise testosterone levels, and shield the testicles from oxidative damage. However, issues related to their limited bioavailability, rapid metabolism, and low solubility make it challenging to use rutin and quercetin in therapeutic settings. It is imperative to use cutting-edge techniques, such as delivery systems based on nanotechnology, to address these issues [5]. By precisely and carefully delivering the medications to the intended site, nanotechnology offers a ground-breaking way to increase the therapeutic efficacy of quercetin and rutin. By encasing flavonoids in nano-sized carriers such as liposomes, nanoparticles, or nanoemulsions, researchers can overcome the basic limitations of flavonoids and improve their pharmacokinetic properties [6]. Furthermore, the simultaneous administration of quercetin and rutin along with synergistic agents or therapeutic payloads is made possible by nanotechnology, which improves the therapeutic efficacy of these compounds against metabolic syndrome and testicular dysfunction [7]. Testicular dysfunction and metabolic syndrome are associated with oxidative stress, which is brought on by an imbalance between the body's

defences against reactive oxygen species (ROS) and their production [12]. Elevated concentrations of malondialdehyde (MDA) in the testicles indicate the existence of oxidative damage and lipid peroxidation. Conversely, the body's defences against oxidative stress are weakened by decreased activity of antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPX), and superoxide dismutase (SOD). Damage to the testicles and issues with sperm production may result from this [13]. Hormonal regulators, including gonadotropin-releasing hormone (GnRH), gonadotropin-inhibitory hormone (GnIH), and kisspeptin (Kiss-1), have important functions in controlling the HPG axis and reproductive function. Imbalances in these hormones, as seen in metabolic syndrome, can disturb the release of gonadotropins, hinder the functioning of Leydig cells, and decrease the generation of testosterone, resulting in testicular dysfunction [14]. Moreover, changes in gastrointestinal peptides such as glucagon-like peptide (GLP) might impact the release of insulin, energy metabolism, and the signaling of reproductive hormones, so establishing a connection between metabolic disruptions and testicular failure [15]. The hormones follicle stimulating hormone (FSH) and luteinizing hormone (LH) control the production of sperm and steroids, respectively. The anterior pituitary gland produces LH and FSH. GnRH, produced by the hypothalamus, stimulates the production of these two hormones. The regulation of spermatogenesis by LH, FSH, and testosterone is widely understood. Testosterone is essential for the proper completion of the spermatogenesis process. In the absence of it, the process of transforming spherical spermatids into spermatozoa during spermiogenesis is hindered. FSH is crucial in both the conversion and differentiation of spermatogonia into spermatocytes. Due to the absence of receptors for testosterone and FSH in germ cells, these hormones must exert their effects on the Sertoli cells, which play a crucial role in supporting and nourishing the germ cells. The manufacture of testosterone is initiated in the Leydig cells upon receiving the signal from LH. The hypothalamus-pituitary-gonadal axis refers to the interconnected route involving the hypothalamus, pituitary gland, and gonads. The hormone Prolactine, which is produced by the anterior pituitary gland, can have a negative impact on male fertility when its levels exceed the normal physiological range [16]. Our study aims to evaluate the efficacy of niosomes loaded with rutin and quercetin in treating rats with testicular dysfunction caused by metabolic syndrome. This study offers insightful information about novel treatment modalities for the treatment of metabolic syndrome-related testicular dysfunction.

2 Materials and methods

2.1 Preparation and Characterization of Niosomes

Initially, the ethanol injection approach was used to create niosomes that contained quercetin and rutin. Particle size, polydispersity index, zeta potential, encapsulation efficiency, drug loading efficiency, scanning electron microscopy, in vitro release research, and stability study were among the criteria used to evaluate the generated systems [17].

2.2 Animals and experimental design

Ninety adult male albino rats in total were acquired from the Faculty of Veterinary Medicine-Zagazig University's animal facility. The rats weighed 180–200 grammes on average when they were 8–10 weeks old. The Zagazig University institutional committee for animal care and use accepted the study, and the rats received standard care in compliance with laboratory animal protocols (approval number: ZU-IACUC/3/F/76/2022). After acclimatisation lasting one week, rats were randomly assigned to six equal groups.

2.3 Induction of obesity induced testicular dysfunction.

To induce obesity induced-testicular dysfunction in the rats, a high-fructose diet was fed to them for ten weeks. Fructose accounts for 60% of the total calories in a high fructose diet. It is made by supplementing a standard mouse meal with 60% weight/weight fructose [18]. After delivering a high-fat diet for 10 weeks, body weight alterations and a significant increase in fasting serum glucose, insulin, and triglycerides were used to demonstrate the presence of fructose-induced metabolic syndrome. After treatment, rats were used for a further four weeks. Group I, the control group, Group II: designated as the obesity group, Group III: obese rats was treated with QU-NIO (0.3 mg/kg b.wt/day) orally on a prophylaxis regimens (QU-NIO was concurrently administered with the HF/FD), Group IV: obese rats was treated with RU-NIO (0.3 mg/kg b.wt/day) orally on a prophylaxis regimens, Group V: obese rats was treated with QU-NIO orally on a therapeutic regimens (QU-NIO was administered after confirmation of the onset of the obesity-induced testicular dysfunction, and Group VI: obese rats was treated with RU-NIO orally on a therapeutic regimens.

2.4 serum and tissue samples preparation

Following an 8-week intervention period and a 12-hour fasting period, each group's rats were euthanized by intraperitoneal injection of sodium thiopental at a dose of 60 mg/kg. Blood samples were taken after four weeks of intervention to validate certain biochemical tests. Afterwards, cardiac punctures were used to extract blood samples, which were then allowed to clot before being centrifuged for 15 minutes at 3,000 rounds per minute. Before being used for biochemical analysis, the serum samples were kept at a temperature of -20 °C for four and eight weeks, respectively. Hypothalamus and testicular tissues were collected and warped in aluminum foil and stored at -80°C until the biochemical and hormonal assays were performed.

2.5 Evaluation of biochemical parameters

Enzymatic colorimetry was carried out using an automated biochemistry analyzer, specifically the Cobas® 6,000 analyzer from Roche Diagnostics Ltd. in Switzerland. This technique was used to

assess a number of parameters, prolactin, FSH, LH, and tesetosterone (free, total) according the manufacturer instruction (MyBioSource, USA).

2.6 Evaluation of oxidative stress markers.

Using a commercial kit (Biodiagnostic, Giza, Egypt), the testicular levels of MDA, SOD, GPX, and CAT in the hepatic and testicular homogenate were measured in accordance with the manufacturer's instructions. A spectrophotometer (Spectronic 3,000 Array, Germany) was used for the measurements.

2.7 Statistical analysis

The standard error of the mean (SEM) ± mean was used to present the data. GraphPad Prism®, Version 9.2, a programme created in San Diego, California, by GraphPad Software Inc., was used to do statistical analysis. The data were analysed using an ANOVA test to compare means and then Tukey-Kramer multiple comparisons tests because the data showed a normal distribution. Statistical significance was defined as a p-value of less than 0.05.

3. Results

3.1 Effect of QU-NIO and RU-NIO on GLP-1 and testicular oxidative stress of the obesity induced-testicular dysfunction rat model.

The results showed a significant ($p < 0.0001$) increased levels in MDA in treated and protective groups of QU-NIO and RU-NIO than control group, but MDA level was decreased in comparison with obese group when used QU-NIO and RU-NIO in protective and treated groups. GLP-1, SOD, GPX, and CAT 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 1).

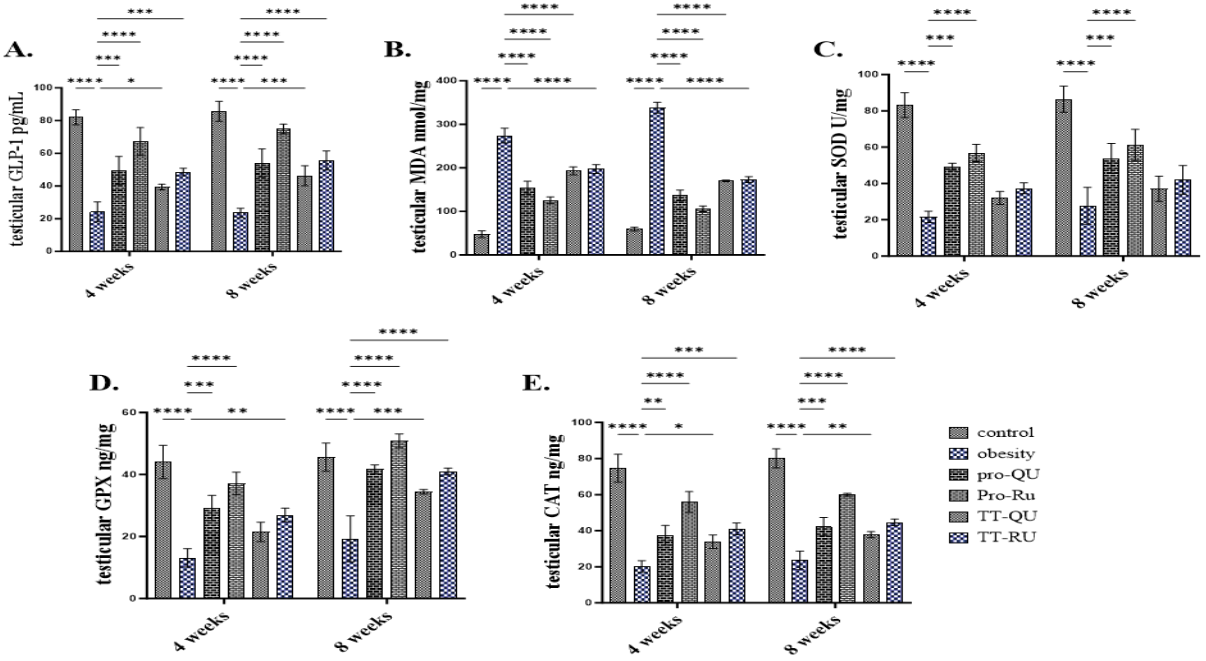

Figure 1. Effect of QU-NIO and RU-NIO on Inflammatory mediators and testicular oxidative stress markers. Data are expressed as means ± SEM. * p < 0.05=mild significant, ** p < 0.01=moderate significant, *** p < 0.001, *** p < 0.0001, indicate highly significant difference.

3.2 Effect of QU-NIO and RU-NIO on testicular oxidative stress of the obesity induced-testicular dysfunction rat model.

The results showed a significant ($p < 0.0001$) decrease levels in FSH, LH, and tesetosterone (free, total) in treated and protective groups of QU-NIO and RU-NIO than control group, but prolactin level was decreased in comparison with obese group when used QU-NIO and RU-NIO in protective and treated groups. The best results were detected in QU-NIO and RU-NIO protective groups than in treatment groups (Figure 10).

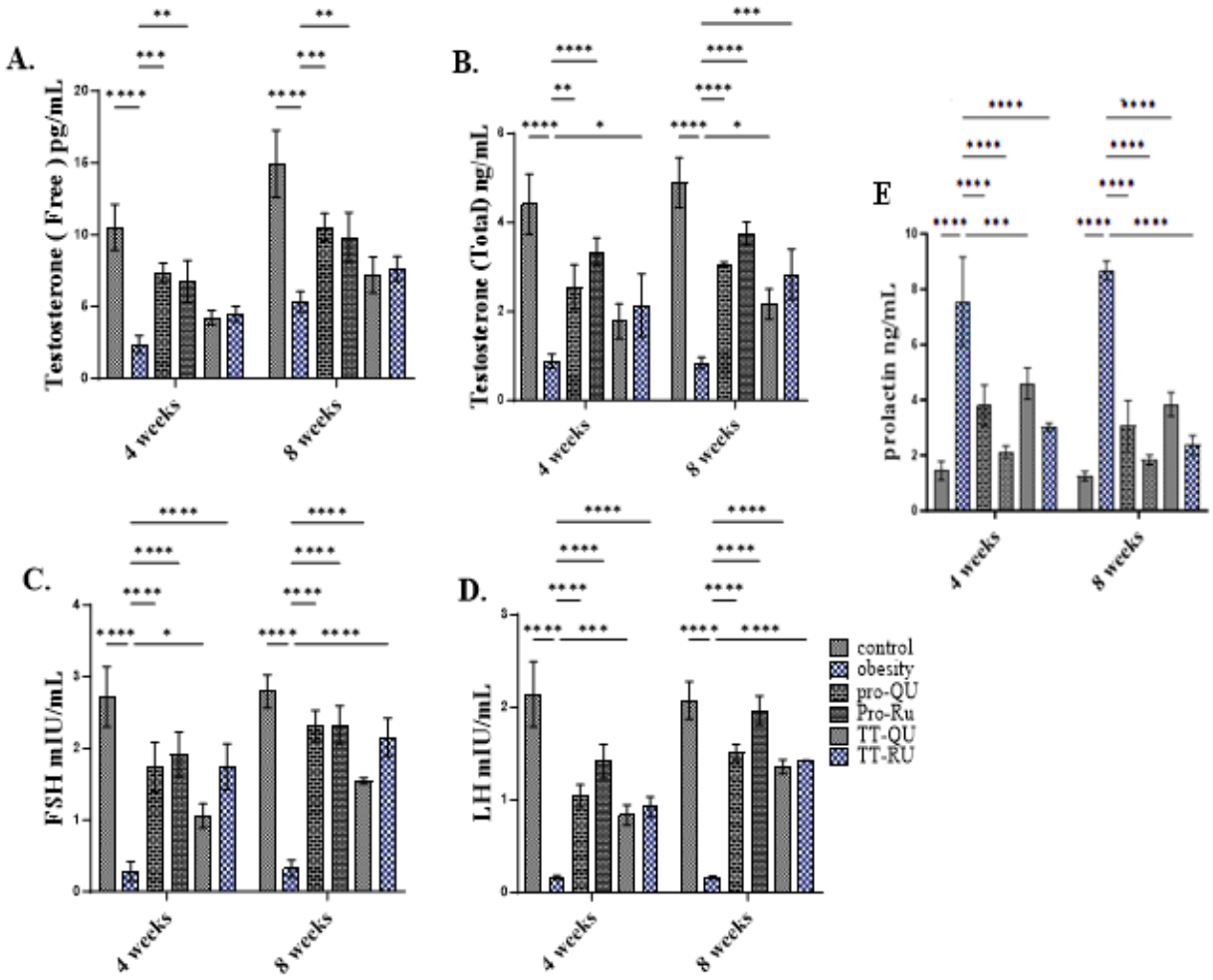

Figure 2. Effect of QU-NIO and RU-NIO on sex hormones. Data are expressed as means ± SEM. * p < 0.05=mild significant, ** p < 0.01=moderate significant, *** p < 0.001, *** p < 0.0001, indicate highly significant difference.

Discussion

Metabolic syndrome (MetS) is a worldwide health concern that can lead to the development of metabolic disorders, cardiovascular and cerebrovascular diseases, which are currently among the most common causes of death, as well as other metabolic conditions. As a result, substantial efforts are made to reduce the incidence and course of MetS through both non-pharmacological and pharmacological means [21]. In this study, we investigated the effects of the natural compound rutin and the quercetin-loaded niosome on a condition that was induced by a high-fructose diet in rats. Our findings supported our hypothesis that the administration of rutin and/or the quercetin-loaded niosome can improve obesity induced-testicular dysfunctions. The process of encapsulating phytochemicals into polymeric nanoparticles or conjugating them to polymers enhances their water solubility and stability, as well as their prolonged and regulated release rate and bioavailability. Drug delivery is greatly enhanced by polymeric nanoparticles

because of their special surface chemistry, which allows them to adsorb, entrap, or covalently attach therapeutic phytochemicals [22]. To achieve a very efficient drug delivery vehicle, it is crucial to characterise the drug-loaded nanocarriers. The therapeutic carrier system's physicochemical characteristics may be thoroughly examined thanks to this characterisation method, which also makes it possible to keep a close eye on how the system behaves at the intended site of action. In this case, the drug release profile, physical stability, cellular absorption, in vivo bioavailability, and bio-distribution of the drug-loaded nanocargo are all directly impacted by the size distribution [23].

Eating a diet high in fat has been shown to cause oxidative stress, which is present in most experimental models and patients with real-world diseases [32]. Presently, there was a drop in TAC and an increase in MDA, the lipid peroxidation product, in obese rats. Our results are consistent with previous research showing that prolonged consumption of a high-fat diet may result in decreased testicular antioxidant defences in obese animals [33]. According to the current research, oxidative stress occurs in untreated obese rats because of an imbalance between the creation of free radicals and antioxidant levels, which lowers the antioxidant defence system. Damage from lipid peroxidation results from the antioxidant defence system being weakened in obese mice. Mice given a high-fat diet showed increased amounts of thiobarbituric acid reactive chemicals [24], which are known to be indicators of lipid peroxidation. This was discovered by Ahmed and colleagues. Eating a lot of calories is a major factor in the lowering of the mitochondrial membrane's flexibility and the encouragement of ROS generation. As a result, oxidative stress may arise from an increase in ROS concentration that exceeds the body's antioxidant defences.

Numerous studies have shown that quercetin-loaded nanoparticles have stronger antioxidant and anti-radical capabilities than quercetin in its free form. The encapsulation of quercetin within polylactic acid nanoparticles (PLGA-NPs) improved both its antioxidative and lipid oxidation inhibitory properties. Moreover, the anti-radical and antioxidant qualities of Qu were enhanced upon its integration into aminoalkyl methacrylate co-polymers, namely poly vinyl alcohol NPs (nanoparticles) [35]. Our findings supported these studies by showing that Qu NPs were very successful in restoring the proper balance between oxidants and antioxidants in the testicular tissue of the obese animals. It has been confirmed by [36].

GLP-1 is a hormone called an incretin that is released by cells in the intestines known as L cells. GLP-1 is a crucial hormone in managing diabetes. Its main functions include promoting the release of insulin, improving the activity of β-cells, and reducing the secretion of glucagon and the synthesis of glucose in the liver. The findings of a study strongly indicate that enhanced β-cell function is a key factor in the hypoglycemic effects of consuming rutin consistent with our study [43]. The GLP-1- Kisspeptin -GnRH signaling pathway has a crucial role in the

development of male testicular dysfunction generated by metabolic syndrome. GLP-1 receptors are found in the hypothalamus and pituitary gland, and they have a crucial role in controlling reproductive function. Kisspeptin, a neuropeptide synthesized by hypothalamic neurons, plays a crucial function in the modulation of gonadotropin-releasing hormone (GnRH) release [44-45]. The pituitary gonadotropic hormones were augmented, leading to a subsequent rise in testicular hormones. This was evidenced by a notable elevation in serum levels of LH, FSH, and testosterone. Comparable findings were derived from certain studies [46-47].

An observed increase in endogenous GLP-1 levels in mice treated with rutin is a noteworthy finding. Rutin acts as a DPP4 inhibitor and GLP-1 secretagogue. Additionally, leptin has been found to promote GLP-1 secretion through the leptin receptor signaling pathway. Thus, the increase in GLP-1 levels may have multiple causes. It is worth noting that rutin-stimulated GLP-1 secretion does not happen through TAS2R. However, the fact that inhibiting TAS2R enhances rutin-induced GLP-1 secretion implies that there is some interaction between rutin's effects and the pan-TAS2R inhibitor probenecid. Rutin and its derivatives have been demonstrated to have an impact on the regulation of intracellular calcium and Ca2+/calmodulin-dependent kinase II during the process of insulin and GLP-1 production [48]. Further research is required to clarify the specific mechanisms by which rutin stimulates the GLP-1 effect.

Our study introduces a new method by using niosomes laden with rutin and quercetin to specifically target the GLP-1-Kisspeptin-GnRH signaling pathway, which has not been investigated in previous research. The lack of previous research addressing these particular findings emphasizes the originality and importance of our study, showing the possibility of revolutionary progress in the treatment of testicular dysfunction linked with metabolic syndrome. This novel approach shows potential in treating testicular dysfunction caused by metabolic syndrome by utilizing the combined therapeutic capabilities of these bioactive chemicals and their interaction with crucial hormonal regulators.

5. conclusion

Overall, our research shows that rutin and quercetin-loaded niosomes may be a useful treatment option for treating metabolic syndrome-related testicular dysfunction. The comprehensive evaluation of oxidative stress markers, GLP-1, and sex hormones highlights the various benefits of niosomal delivery systems in focusing on critical pathways implicated in obesity-induced testicular failure. These promising results support the need for more investigation and clinical trials to verify the safety and efficacy of niosomes loaded with rutin and quercetin as a possible treatment for testicular dysfunction in individuals with metabolic syndrome.

6. Limitation of the current study

Further investigation is required to elucidate the long-term effects and potential adverse reactions of niosomes loaded with rutin and quercetin in different animal models and human participants suffering from testicular dysfunction due to metabolic syndrome. To maximise treatment efficacy and minimise potential side effects, it is imperative to investigate the optimal dosage, frequency, and duration of quercetin- and rutin-loaded niosome therapy. The effective implementation of this therapy in clinical settings depends on the results of this research. Thorough clinical trials that evaluate the safety, efficacy, and long-term benefits of rutin and quercetin-loaded niosomes in improving testicular health and reproductive outcomes for individuals with metabolic syndrome are required before implementing the promising preclinical results of these therapeutic agents into actual medical practice 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. Mohamed Hamza supervise the current work.

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