Al-Qadisiyah J. Vet. Med. Sci. 2025; 24 (1). 10.29079/qjvms.2025.159949.1085

ISSN P: 1818-5746
E: 2313-4429
qjvms.qu.edu.iq

The effect of nano lutein compound on the male reproductive system of white rats exposed to hydrogen peroxide induced oxidative stress

Ali abdullah. AL-dhalimy, Ahmed J. AL-Naely*
*Department Biology, College of Education, University of Al-Qadisiyah, Iraq.
*Email: edu.bio.posta110@qu.edu.iq
*E-mail: ahmed.jassem@qu.edu.iq

Submitted: May 07, 2025
Revised: May 21, 2025
Accepted: June 01, 2025

Abstract Forty-eight male albino rats, aged 12–14 weeks and weighing 200–250 g, were used to assess the protective nano lutein effects on the male reproductive system from oxidative stress conditions. The experiment, conducted in the animal house of the Department of Biology, College of Education, University of Al-Qadisiyah, consisted of dividing the rats into six groups (n = 8 per group): a control group administered drinking water only; T1, administered 1% hydrogen peroxide (H_2O_2); T2, treated with normal lutein (48 mg/kg); T3, treated with nano lutein (24 mg/kg); T4, administered H_2O_2 and normal lutein; and T5, treated with H_2O_2 and nano lutein. Experimental period was 45 days.Results revealed that there was a significant reduction ($P < 0.05$) in serum testosterone, LH, and FSH levels of T1 rats treated with H_2O_2 only, as well as sperm concentration, motility, viability, and normal morphology. However, treatments T2 and T3 were not different from the control group, suggesting that lutein—normal and nano—did not exert any adverse effect on reproductive parameters. Interestingly, T4 and T5 groups (treated with H_2O_2 and either form of lutein) exhibited significant ($P < 0.05$) improvement in hormone profile and sperm quality when compared with T1, indicating a protective effect. Although T5 showed a greater improvement in results, the difference between T4 and T5 was not significant. The findings support nano lutein's antioxidant action in inhibiting oxidative damage in male reproductive function.

Keywords: nano lutein compound; oxidative stress; male reproductive system; sperm parameters

Introduction

Oxidative stress is recognized as one of the causes of infertility through damaging sperm functioning, it is a condition characterized by enhanced cell damage owing to the build-up of reactive oxygen species (ROS) (1). Increased ROS level has a harmful impact on sperm quality and diminished fertilizing ability (2). ROS are known to attack and modify sperm DNA, proteins, and lipids, where they interfere with the enzymatic systems and cause irreversible cell death. this action is an aggregate sense which causes decrease in sperm fertility parameters (3).Sperm membrane lipid peroxidation and free radical production adversely affect sperm motility and morphology which compromising sperm function and ultimately decreasing fertility(4) .polyphenols, flavonoids, terpenoids and carotenoids are a diverse of compounds found in nature called plant biologically active compounds(5). These are phytochemicals or secondary metabolites have antioxidant and anti-inflammatory activity, so they are required in nutrition for their therapeutic and protective activity (6). Carotenoids are a group of lipophilic have yellow and orange pigments, found in nature ubiquitously and cannot be synthesized by humans and animals, so they must be acquired through food intake(7). Lutein (LUT) is a lipid-soluble xanthophyll carotenoid have healthy properties, found commonly in dark-green leafy vegetables, spinach, parsly, kale and egg yolk(8). They commercially synthesized from marigold flour (Tagetes erecta), have chemical formula C40H56O2 with molecular weight 568.871 g/mol.(9).As a potent antioxidant, lutein has been proven to attenuate oxidative stress and free radicals-highly chemically reactive species that start a chain of lipid peroxidation leading to the oxidization of cell membrane lipids (11 ,10) .

Materials and Methods

Ethical approval

The current study procedures were approved by the College of education, University of AlQadisiyah.

Experimental Design

Lutein Compound

Al-Qadisiyah J. Vet. Med. Sci. 2025; 24 (1). 10.29079/qjvms.2025.159949.1085
ISSN P: 1818-5746
E: 2313-4429
qjvms.qu.edu.iq

Lutein (Newgate/Britain) was referenced from pharmaceutical stores in Al-Muthanna province in the form of a bottle containing 30 tablets of dose 50 mg/kg. The dose of regular lutein taken in the experiment was selected based on a previous study by (12), in which an effective dose was found to be 48 mg/kg of body weight, with a very effective impact on most physiological functions, and, after that, the lutein was finely grind using an electric grinder. The dosage of nano-lutein in was determined to be 24 mg/kg.

Experimental Animals

A total of eight adult male laboratory rats were purchased from the College of Veterinary Medicine, University of Kufa. They were 12–14 weeks old and weighed between 200 and 250 g. These animals were fed two weeks for acclimatization and checking their health condition. The study was conducted in the animal house of the Department of Biology, College of Education, University of Al-Qadisiyah.

Parameters Studied

Hormonal changes were evaluated through serum testosterone, LH, and FSH levels. Sperm concentration, viability, morphology and motility were sperm parameters that changed.

Experimental Design

48 rats were randomly divided into six groups of 8 rats, and the study lasted for 45 days as follows:

- Control Group: During the 45-day experimental period, rats were given normal drinking water.
- Group 1 (T1): watered by 1% hydrogen peroxide in drinking bottles for the duration of the experiment.
- Group 2 (T2): watered by lutein in a dose (48 mg/kg/ body weight.) throughout the experiment.
- Group 3 (T3): Nano-lutein watered in a dose (24 mg/kg body weight) during the days of the experiment.
- Group 4 (T4): watered by 1% hydrogen peroxide + normal lutein at 48 mg/kg during the experimental period.
- Group 5 watered by 1% hydrogen peroxide + nano-lutein in a dose 24 mg/kg body weight during the 45-day experimental period.

Statistical analysis

After collecting and tabulating the data, the SPSS V.25 statistical analysis program was used. The data were statistically analyzed according to the One-Way ANOVA test, and the means of the experimental groups were compared when the differences between them were significant using the Least Significant Difference (LSD) test at a significance level of 0.05.

Results

Table 1 showed significant (P<0.05) decrease in serum Testosterone, FSH and LH levels in T1 group when compared with control and the other groups, also the results showed that there is no significant deference between T2 and T3 groups, and the control when compared, even that there is a statistical increase but without significancy. On the other hand groups T4 and T5 showed a lower statistical significant (P<0.05) deference of serum hormone levels when compared with the control, and higher statistical significant (P<0.05) deference when compared with T1 group. The results also showed that there is no statistical significant deference in serum hormone levels between T4 and T5.

Table (1) Role of regular and nano-lutein compounds in the average concentrations of testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in male white rats exposed to oxidative stress induced by hydrogen peroxide.

Group	Parameters		
	Testosterone (ng/ml)	FSH (IU/L)	LH (IU/L)
C	2.62±0.12 AB	2.74±0.76 A	3.86±0.20 A
T1	0.83±0.23 D	0.90±0.80 C	1.02 ±1.48 C
T2	3.17±0.97 A	2.93±1.35 A	4.13±1.10 A
T3	3.22±0.41 A	3.13±0.50 A	4.20±0.66 A
T4	1.83±0.11 C	1.83±1.06 B	2.56±1.40 B
T5	2.05±2.02 BC	1.79±0.08 B	2.81 ±0.76 B
LSD	0.76	0.88	0.90

Values are expressed as mean ± SE.
different superscript letters in the same column differ significantly between treatments at P 0.05.

C: control group – animals were treated with normal drinking water during the whole 45-day experimental period.

T1: First treatment – the animals were subjected to oxidative stress by administration of 1% hydrogen peroxide through 45 days from drinking bottles.

T2: Animals given regular lutein at a dose of 48 mg/kg body weight for 45 days.

T3: Third treatment – animals received nano-lutein at a dose of 24 mg/kg body weight for 45 days.

T4: Fourth - regular lutein (48 mg/kg) with 1% hydrogen peroxide (45 days).

T5: 5th treatment - animals were given nano-lutein (24 mg/kg) and hydrogen peroxide 1% for 45 days.

Al-Qadisiyah J. Vet. Med. Sci. 2025; 24 (1). 10.29079/qjvms.2025.159949.1085
ISSN P: 1818-5746
E: 2313-4429
qjvms.qu.edu.iq

Changes in Sperm Parameters

The data in table (2) show the effect of normal lutein & nano-lutein compounds on sperm parameters in male white rats. The results revealed that the first treatment group (T1: hydrogen peroxide 1%) had a significantly lower ($P < 0.05$), as compared to control (C) and other groups.

Treatment T4 had an overall significant lower number ($P < 0.05$). Furthermore, T5 resulted in significantly improved sperm parameters compared to T2, T3, but there is no significant difference between T4 and T5, although Treatment T5 showed the visible improvement of sperm parameters, which was administered hydrogen peroxide and nano-lutein.

Effect of Regular and Nano-Lutein Compounds on Sperm Parameters in Male White Rats Exposed to Oxidative Stress Induced by Hydrogen Peroxide (Sperm Concentration, Percentage of Viable, Morphologically Normal, and Motile Sperm)

Group	Parameters			
	Sperm Concentration (Million/ml)	Viable Sperm (%)	Normal Sperm (%)	Motile Sperm (%)
C	85.66±2.90 A	83.16±3.32 AB	81.66±1.76 AB	81.83±1.58 AB
T1	42.66±2.60 C	36.66±2.33 D	38.91±2.23 D	33.33±2.96 D
T2	90.00±2.08 A	84.50±1.80 AB	88.08±1.15 A	86.00±1.52 A
T3	91.33±2.02 A	88.16±1.36 A	88.33±1.85 A	87.50±1.80 A
T4	65.66±4.05 B	59.33±3.52 C	61.16±3.46 C	58.50±3.61 C
T5	71.33±2.02 B	68.56±2.02 BC	69.08±2.46 BC	67.50±2.17 BC
LSD	6.48	19.06	15.51	17.36

The same information mentioned below Table (1)

Discussion

Hormonal Changes

Results of statistical analysis demonstrated that (T1) (exposed to oxidative stress) was significantly ($P<0.05$) with a decrease in serum concentrations of testosterone, LH, and FSH as depicted in Table (1). These findings were consistent with the study done by (13). Testosterone is essential for male puberty, sperm production, and testicular growth, but its synthesis and secretion are controlled by the pituitary hormones FSH and LH (14).Testosterone secretion has been found to decrease due to the effect of oxidative stress, such as hydrogen peroxide, on the physiological of hypothalamic-pituitary-gonadal (HPG) mechanism by reducing the secretion of FSH and LH. The stimulation and differentiation of Leydig cells, the primary function of luteinizing hormone (LH) (15). Alternatively, this disruption may also work indirectly by reducing pituitary axis sensitivity by negative feedback, resulting in an increase in CRH stimulation of the adrenal medulla through a PKC-dependent pathway (16). It is capable of inhibiting the HPG axis, thus ultimately decreasing the release of GnRH from the hypothalamus (17).The decline could be due to a decrease in the release of gonadotropin-releasing hormone, or an impaired response of the pituitary to release this hormone. Moreover, decreased antioxidant defense mechanisms and enhanced free radical production are well-established contributors to gonadotropin inhibition, which— in turn— ultimately modulates reproductive hormones (18).However, there was an increase in the hormone levels in the T3 and T4 treated groups (non-significant $P>05$) when compared to control (C) and other treated groups. natural and nano-lutein treated groups (T2 and T3) showed appreciable enhancement of hormonal concentration. However, Treatments T4 and T5 exhibited a significant increase ($P<0.05$) in serum testosterone, LH, and FSH levels as compared to T1, which was additionally subjected to oxidative damage. Nonetheless, we neither observed a clear difference between T4 and T5 despite the apparent strong response of (T5). This enhancement can be attributed to the possession of carotenoids such as lutein which guard reproductive tissues and energies against oxidative damage induced by free radicals and enhance their fantastic-dimensional potency. Lutein may also stimulate the release of pituitary hormones (LH, FSH)

Al-Qadisiyah J. Vet. Med. Sci. 2025; 24 (1). 10.29079/qjvms.2025.159949.1085
ISSN P: 1818-5746
E: 2313-4429
qjvms.qu.edu.iq

that further generate sex hormones (19).Additionally, The same research has also been observed with a diet supplemented with 25, 50, 75 mg/kg lutein in improving the steroid hormones in aging rosters. Aging is associated with a decrease in Leydig cell testosterone production, whereas lutein supplementation improved semen quality, preserved reproductive hormone levels, and lowered oxidative products. Thus lutein could be a dietary supplement to improve the fertility and maintain normal spermatogenesis while preventing the age-associated degenerative disorders in aged roosters. (20).A study by (21) also showed cyclosporin-induced reproductive toxicity protection by lutein in male rats via modulating hormones and androgenic enzymes and maintaining antioxidant and steroidogenic enzyme activities in the testes to promote testosterone synthesis. These enzymes are biomarker candidates for increased testosterone levels, which vary in importance for testicular development and function.Hormonal parameters in (T5) were not significantly different from control group and were closest among all treatments exposed to oxidative stress. This may be related to the potential of nano-lutein, in line with the results of (22) who found that serum nano-lutein levels were 28% and 31% higher following ingestion of normal lutein versus equivalent doses of nano-lutein, respectively. Regular lutein is several times less absorbable than nano-lutein, as its surface area-to-volume ratio is drastically smaller.

Changes in Sperm Parameters

Table (2) demonstrates significant effect in total sperm count, motility, viability and normal morphology in the cauda epididymis of rats in (T1) when compared to control group (C) and other treatment indicating oxidative stress induction. Administration of hydrogen peroxide decreased sperm motility, concentration and viability and increased the percentage of abnormal sperm significantly. These results are consistent with those of (23, 24). It matters because 46% had low count and 69% were found to have low motility in the study (2024), and they also identified a 50% decrease in overall spermatozoa parameters, as well as a 21% increase in the prevalence of abnormal forms among these individuals compared to controls.Infertile males with high free radical production, particularly reactive oxygen species (ROS), exhibit sperm damage in the form of structural abnormalities and decreased motility. As oxidative stress is a significant cause of decrease fertility (25, 26). showed that ROS decreases sperm count as well as sperm viability. This could also be due to a decrease in the number of Leydig cells and necrosis of those cells, This reduction interferes with testosterone synthesis, a hormone necessary for spermatogenesis and overall sperm quality. Testosterone plays a supportive role in the spermatogenic process hence lower levels are associated with lower sperm concentrations in the epididymis. Low testosterone levels have been associated with a sharp decrease in epididymal sperm count, and a deficiency in testosterone is known to reduce sperm functional efficiency(27).Further investigation showed increased levels of tumor necrosis factor-alpha (TNF-α) and decreased levels of the anti-apoptotic protein Bcl-2, which may be indicative of oxidative stress-induced acceleration of testicular cell apoptosis, another contributor to the decrease in sperm count. This results in decreased reproductive hormones (testosterone, LH and FSH) with subsequent necrosis and atrophy of the testicular cells, thus interfering with spermatogenesis. As a result, it decreases sperm count, motility, and viability (28). One of the key factors affecting sperm quality is the sperm motility, the dysfunction of which plays an important role in the generation of infertility (29). High ROS concentrations correlate with sperm inactivity and have been shown to reduce sperm motility, as hydrogen peroxide, which is one of the main members of oxidative by-products, can quickly enter into the sperm membrane and inactivate important enzymes that are involved in the locomotive aspect of sperms (23). In the cauda epididymis, T2 treated with natural (0.05 mg/kg BW) lutein and treatment T3 treated with nano lutein (0.05 mg/kg BW) exhibited significant improvement in total sperm count, motility, viability, and morphology as compared to those of control and other treatments. Similarly, T4 and T5 exhibited a marked improvement over T1, which was subjected to oxidative stress. Notably, Treatment T5 demonstrated the most pronounced improvements when nano-lutein was co-treated with hydrogen peroxide. This could be as a result of the rewarding on the anti-oxidant properties of carotenoids (lutein collectively) and their ability for retaining cellular integrity (19). also noted that carotenoids improve male reproductive function, sperm motility, viability, and concentration, as well as significantly reduce MDA levels. Carotenoids exhibited anti-oxidant activity and improved fertility and spermatogenesis.These results align with those reported by (30). In this study, male mice exposed to nano-titanium dioxide particles were simultaneously treated with lutein at 5–10 mg/kg for 35 days. Their study showed that lutein protected spermatozoa, increased motility and activity, preserving membrane integrity, supported mitochondrial function, induced testosterone levels and anti-oxidant capacity. Hydroxyl radicals and other toxic oxidative chemicals (hydrogen peroxide and reproductive toxins) were clamped by lutein.The increase in sperm concentration observed in the present study could be attributable to elevated levels of

testosterone and FSH in the groups that received a treatment with normal and nano-lutein in the presence of hydrogen peroxide. The initiation and completion of spermatogenesis are directly affected by these hormones (31). Testosterone is crucial for sperm cell differentiation and, in conjunction with FSH, aids in the maturation of sperm cells (32). Interestingly, treatment (T5) that was oxidatively stressed and treated with nano-lutein and hydrogen peroxide presented with a greater number of motile sperm that had typical morphology relative to the control (C); however, the differences were not statistically significant ($P < 0.05$). The bioavailability of lutein can be improved through nano-encapsulation using non-soluble PCA and such various nanomaterials thus maximizing its therapeutic efficacy. Compared to the free lutein, nano-lutein has a better release profile because free lutein has poor solubility and limited stability(33)

Conclusion

White rats receive nano lutein with remarkable potency against the damaging effects of oxidative stress on the male reproductive system hormonal parameters and changes in sperm parameters. The increased bioavailability and higher tissue penetration may explain its superior activity as compared to normal lutein. Thus, nano lutein may become a useful therapeutic anti-oxidant agent in the treatment of disorders caused by oxidative stress.

Acknowledgement

The research study was self-funded only.

Conflict of interest

No conflict of interest is found for the present study.

Funding source

This research had no specific fund; however, it was self-funded by the authors.

References

1. Abdulla JM, Al-Okaily BN. Histomorphometric and histopathological alterations of rat testis following exposure to hydrogen peroxide: Protective role of resveratrol supplement. The Iraqi Journal of Veterinary Medicine. 2022;46(1):17-23.https://www.jcovm.uobaghdad.edu.iq/index.php/Iraqijvm/article/download/1313/1565
2. Moazamian R, Polhemus A, Connaughton H, Fraser B, Whiting S, Gharagozloo P, et al. Oxidative stress and human spermatozoa: diagnostic and functional significance of aldehydes generated as a result of lipid peroxidation. MHR: Basic science of reproductive medicine. 2015;21(6):502-15.https://doi.org/10.1093/molehr/gav014
3. Agarwal A, Virk G, Ong C, Du Plessis SS. Effect of oxidative stress on male reproduction. The world journal of men's health. 2014;32(1):1.https://doi.org/10.5534/wjmh.2014.32.1.1
4. Pavuluri H, Bakhtiary Z, Panner Selvam MK, Hellstrom WJ. Oxidative Stress-Associated Male Infertility: Current Diagnostic and Therapeutic Approaches. Medicina. 2024;60(6):1008.https://doi.org/10.3390/medicina60061008
5. Lojek A, Denev P, Ciz M, Vasicek O, Kratchanova M. The effects of biologically active substances in medicinal plants on the metabolic activity of neutrophils. Phytochemistry reviews. 2014;13:499-510.https://doi.org/10.1007/s11-9340-014-101x
6. Prakash D, Gupta K. The antioxidant phytochemicals of nutraceutical importance. The Open Nutraceuticals Journal. 2009;2(1):35-20.https://benthamopenarchives.com/contents/pdf/TONUTRAJ/TONUTRAJ-2-20.pdf
7. Amorim-Carrilho K, Cepeda A, Fente C, Regal P. Review of methods for analysis of carotenoids. TrAC Trends in Analytical Chemistry. 2014;56:49-73.https://doi.org/10.1016/j.trac.2013.12.011
8. Fuad NIN, Sekar M, Gan SH, Lum PT, Vaijanathappa J, Ravi S. Lutein: A comprehensive review on its chemical, biological activities and therapeutic potentials. 2020.https://www.phcogj.com/article/1291
9. Maoka T. Carotenoids as natural functional pigments. Journal of natural medicines. 2020;74(1):1-16.https://doi.org/10.1007/s11418-019-01364-x
10. Torregrosa-Crespo J, Montero Z, Fuentes JL, Reig Garcia-Galbis M, Garbayo I, Vílchez C, et al. Exploring the valuable carotenoids for the large-scale production by marine microorganisms. Marine Drugs. 2018;16(6):203.https://doi.org/10.3390/md16060203
11. Wisniewska-Becker A, Nawrocki G, Duda M, Subczynski W. Structural aspects of the antioxidant activity of lutein in a model of photoreceptor membranes. Acta Biochimica Polonica. 2012;59(1):119-24.https://www.frontierspartnerships.org/articles/10.18388/abp.20/2185_12pdf
12. Zhang Y, Ding H, Xu L, Zhao S, Hu S, Ma A, et al. Lutein can alleviate oxidative stress, inflammation, and apoptosis induced by excessive alcohol to ameliorate reproductive damage in male rats. Nutrients. 2022;14(12):2385.https://doi.org/1/0.3390nu14122385

13. Ahmed TS, Thanon RT, Hadi KA. Effect of Graviola (Annona muricata) on Some Physiological Parameters and Fertility of Male Albino Rats Exposed to Oxidative Stress. Egyptian Journal of Veterinary Sciences. 2025;56(6):1307-15.https://doi.org/10.21608/ejvs.2024.272512.1874
14. Andrea S, Giulia R, Hackett G, Seminara SB, Huhtaniemi IT, Rey RA, et al. Paediatric and adult-onset male hypogonadism (Primer). Nature Reviews: Disease Primers. 2019;5(1).https://search.proquest.com/openview/0d5c2f39dda9a8f3524334b711616c02/1?pq-origsite=gscholar&cbl=2069613
15. Skórka-Majewicz M, Goschorska M, Żwierełło W, Baranowska-Bosiacka I, Styburski D, Kapczuk P, et al. Effect of fluoride on endocrine tissues and their secretory functions--review. Chemosphere. 2020;260:127565.https://doi.org/10.1016/j.chemosphere.2020.127565
16. Mazzocchi G, Malendowicz LK, Rebuffat P, Tortorella C, Nussdorfer GG. Arginine-vasopressin stimulates CRH and ACTH release by rat adrenal medulla, acting via the V1 receptor subtype and a protein kinase C-dependent pathway. Peptides. 1997;18(2):191-5.https://doi.org/10.1016/S0196-9781(96)00294-X
17. Raftogianni A, Roth LC, García-González D, Bus T, Kühne C, Monyer H, et al. Deciphering the contributions of CRH receptors in the brain and pituitary to stress-induced inhibition of the reproductive axis. Frontiers in Molecular Neuroscience. 2018;11:305.https://doi.org/10.3389/fnmol.2018.00305
18. El-Demerdash FM, Yousef MI, Radwan FM. Ameliorating effect of curcumin on sodium arsenite-induced oxidative damage and lipid peroxidation in different rat organs. Food and Chemical Toxicology. 2009;47(1):249-54.https://doi.org/10.1016/j.fct.2008.11.013
19. Pasquariello R, Anipchenko P, Pennarossa G, Crociati M, Zerani M, Brevini TA, et al. Carotenoids in female and male reproduction. Phytochemistry. 2022;204:113459.https://doi.org/10.1016/j.phytochem.2022.113459
20. Li Y, Liu N, Wang J. Effect of dietary lutein on semen quality, reproductive hormones, and oxidative injury products of aged roosters. European Poultry Science/Archiv für Geflügelkunde. 2023(390).https://search.ebscohost.com/login.aspx?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=00039098&AN=174429186&h=1c5iDaccCp%2BdddsZuutOKaM9ZkvLV56DcIA%2BF%2BsAt0W56fVbWDjybIYpwJntsOwG%2Bq6J86Ktay%2Fa%2FT2Zvnoa8w%3D%3D&crl=c
21. Oyovwi, , Ben-Azu B, Edesiri TP, Arientare RR, Emojevwe V, et al. Lutein attenuates cyclosporin-induced testicular impairment in male rats through modulation of androgenic hormones and enzymes. Pharmacology and Toxicology of Natural Medicines (ISSN: 2756-6838). 2022;2(1):12-24.https://doi.org/10.52406/ptnm.v2i1.17
22. Vishwanathan R, Wilson TA, Nicolosi RJ. Bioavailability of a nanoemulsion of lutein is greater than a lutein supplement. Nano Biomed Eng. 200-38:(1)1;9 .49http://nanobe.org/Data/View/96?type=100#:~:text=DOI%3A%2010.5101/nbe.v1i1.p38%2D49
23. Pujianto DA, Oktarina M, Sharaswati IAS. Hydrogen peroxide has adverse effects on human sperm quality parameters, induces apoptosis, and reduces survival . Journal of human reproductive sciences. 2021;14(2):121-8.https://journals.lww.com/jhrs/fulltext/2021/14020/hydrogen_peroxide_has_adverse_effects_on_human.4.aspx#:~:text=%D9%8A%D9%88%D9%86%D9%8A%D9%88%202021.%20%7C%20%D8%AF%D9%88%D9%89%3A-,10.4103/jhrs.jhrs_241_20,-%D9%8A%D9%86%D8%B3%D8%AE
24. Mararajah S, Giribabu N, Salleh N. Chlorophytum borivilianum aqueous root extract prevents deterioration of testicular function in mice and preserves human sperm function in hydrogen peroxide (H2O2)-induced oxidative stress. Journal of Ethnopharmacology. 2024;318:117026.https://doi.org/10.1016/j.jep.2023.117026
25. Zini A, Al-Hathal N. Antioxidant therapy in male infertility: fact or fiction? Asian journal of andrology. 2011;13(3):374.https://pubmed.ncbi.nlm.nih.gov215//16118
26. Elmussareh M, Mahrous A, Kayes O. Antioxidant therapy for male subfertility: myth or evidence-based? Trends in Urology & Men's Health. 2015;6(1):35-9.https://wchh.onlinelibrary.wiley.com/doi/pdfdirect/10.1002/tre.439
27. Yang L, Liu S, Song P, Liu Z, Peng Z, Kong D, et al. DEHP-mediated oxidative stress leads to impaired testosterone synthesis in Leydig cells through the cAMP/PKA/SF-1/StAR pathway. Environmental Pollution. 2025;366:125503.https://doi.org/10.1016/j.envpol.2024.125503
28. Mohammad SA, Ramadan BK, Schaalan MF. Retraction: Mitigation of azathioprine-induced

ISSN P: 1818-5746
E: 2313-4429
qjvms.qu.edu.iq

testicular atrophy by taurine; an impact on inflammation, oxidative perturbations and apoptosis. NRC Research Press; 2018.https://doi.org/10.1139/cjpp-2018-0072

29. Dcunha R ,Hussein RS, Ananda H, Kumari S, Adiga SK, Kannan N, et al. Current insights and latest updates in sperm motility and associated applications in assisted reproduction. Reproductive Sciences. 2022:1-19.https://doi.org/10.1007/s43032-020-00408-y
30. Khodadadi R, Jalali A, Moghadasi S, Farahani M. Environmental exposure to titanium dioxide nanoparticles disrupts DAZL gene expression and male reproductive function in mice: Protective role of lutein. Food and Chemical Toxicology. 2025;195:115128.https://doi.org/10.1016/j.fct.2024.115128
31. Stocks BT, Oppenheimer AG, Campbell KJ, Lindsey JP, Kohn TP, Alexander JM, et al. Optimal restoration of spermatogenesis after testosterone therapy using human chorionic gonadotropin and follicle-stimulating hormone. Fertility and Sterility. 2024.https://doi.org/10.1016/j.fertnstert.2024.10.019
32. Porth C. Essentials of pathophysiology: concepts of altered health states: Lippincott Williams & Wilkins; 2011. pp1013. p.https://books.google.com/books?hl=ar&lr=&id=FFg88IaReBwC&oi=fnd&pg=PR19&dq=Porth+C.+Essentials+of+pathophysiology:+concepts+of+altered+health+states:+Lippincott+Williams+%26+Wilkins%3B+2011.+pp1013.+p&ots=nnIhs8rMpk&sig=MnpGWKC9CgF0YK1_276B51pKZic
33. Maghsoudloo M, Bagheri Shahzadeh Aliakbari R. Lutein with various therapeutic activities based on micro and nanoformulations: A systematic mini-review. Micro Nano Bio Aspects. 2023;2(4):1-7.https://doi.org/10.22034/mnba.2023.409671.1041