ISSN NO: 2771-8840
Date of Publication: 20-06-2023

Molecular docking of hydroxytyrosol and its nanoparticles to improvement of reproductive system in male rats

[1]Hussein Ali Khayoon , [2]Malaa m. mohammestaki, [3]Nadya Jamal Ibrahim, [4]Adnan Mansour. [5]Jasim Hassan RajihAlkelaby

[1]Al-Muthanna university College of Nursing, department of basic medical science
[2] University of technology / nanotechnology and advanced research center
[3],[4] College of Veterinary Medicine, AL-Qasim green University Iraq
[5] Department of anesthesia College of hilla university Iraq
Author: hussain.ali@mu.edu.iq

Abstract: The interactions of protein Ligand play important in built of many drugs design, so by Appling: molecular docking, to identify and investigated their affinity of hyroxy-tyrosol in androgen receptor. The structural a three dimensional (3D) of androgen receptor was Protein Data Bank chosen, then 3D docken with of tested compound. The results represent that (hydroxyl-tyrosol) exhibited best binding affinity of −6.2 kcal/ mol with AR followed by anastrazole−5.2 kcal/ mol. A total Thirty Mature Wister male albino rats . Adult Thirty male rats were equally and randomly distributed into 3rd groups of ten animals for each group. The first group, named as negative control (NC), The second group(T1) and (T2) daily orally received HT extract (50 mg/kg as free and nanoparticles respectively. The antioxidant capacity of HT by scavenging of ROS and clear improvement of reproductive function and testicular tissues.

Key words: docking, hyroxy-tyrosol

Introduction

The quality of the semen is one of the most important factors for the success of natural insemination that was closely applied in animals[1]. Spermatogenesis is a process differentiated cell that production of fertilized sperm, which eventually fuse with an egg to form a zygote[2].Shah, Khan [3] confirm thatsertoli cells considered responsible from normal spermatogenesis, which providing nutrients for mitosis and meiosis of male germ cells and cell junctions. Spermatogenesis was regulated by several gene, largely are exclusively expressed in the testis[4]. Nanotechnology is the science and technology of small particles under 100 nm) with new changes in their physiochemical character specially in surface area and solubility[5, 6].Hydroxytyrosol (4-(2-Hydroxyethyl)-1,2-benzenediol) is one of the most phenolic compounds with potent effect approved by European Food Safety authority (EFSA) for medical application[7, 8].HT has been explore versus a number of health problems including diabetes, muscle damage,nervous disorders, inflammation, , angiogenesis, toxicity of heavy metal ,oxidative stress, hemolysis, and nephrotoxicity with positive outcomes[9]. The ultrasound waves in high intensitywith frequencies in the range of 20 to 100 kHz generate cavitation, which is accountable for biomaterial surface damage and internalization of bioactive compounds from the medium into the polymeric substance. The polyphenollic compound of HT extracted from leaves of olive and oil. HT has wonderful activity due its potent anti-inflammatory, antioxidant, and antibacterial [7, 10]. The antioxidant capacity of HT by scavenging oxidant chemical species and increase the antioxidant enzymes expression .Han, Zhong [11]coworker (2021) reported that HT elevated antioxidant molecules plamsa plus testosterone and its derivatives, while diminished bile acids and the derivatives that enhance spermatogenesis and semen quality[12]. The biological HT efficacy and its safety with minor not clear adverse effects even at very high doses—and with this idea , the focus of present study was to explore whether the of HT, to improvement of testicular tissue and sperms quality.

Materials and methods

Choose the receptor and ligaments. The active compound hydroxytyrosol report was considered to study molecular DO : Testosterone, Stanazol, Nanodrolonidecanoate, and testosterone propionat tocopherol were considered to be similar to the study's control totals. Protein detailing. "Androgen receptor (AR)" has

ISSN NO: 2771-8840
Date of Publication: 20-06-2023

been altered by the restoration of structure of crystal of the RCSB PDPtp material AR ligand hydroxytyrosol (PDB: 1E3G) complex://www.rcsb.org/pdb. As mentioned in 23,25.

Experimental design

This study was carried out at, College, Veterinary Medicine, / Al-Qasim Green University. Weighted adult male rats (180 200 g) between 60 and 65 days of age. A total of thirty male of male mice with an average weight of 170 to 190 grams, obtained from the animal house at the Veterinary Medicine College, Qasim Al -Khadra University. Thirty male mice are distributed randomly and uniformly in three groups of ten animals each. The three groups supplied with regular food ,water were exposed to the same ventilation and living conditions. The first group, known as the Negative Check (NC), was the Daily Oral Management Trailer. The second group, called T1, is subject to the management of the HT extract (50 mg/kg)13. The finally of G3 called (T2) drinking a treatment of HT of nanoparticles at (50 mg /kg).

Preparation of hydroxytyrosol - nanoparticles:

Nanoparticles of hydroxytyrosol according to[5, 14]with mild modification. The aqueous solution included 200 mg of hydroxytyrosol(MCE) was dissolved in 1 ml of de-ionized water solvent and incubated overnight, then sonicated for (10 min). Aqueous phase was transfer to An organic phase of hexane(50 ml) , Next, put on a 500 -round magnetic fuss tape for 10 hours until you remove the Hexane belt. Then the percentage 1:1 of 0.05 % of TPGS was we added to the solution for make emulsifier solution. The emulsified hydroxytyrosol solution was directly transferred to the prob-sonicater(ninemin, pulse on time fifteen seconds with pulse off time15seconds) and 50% amplitude .

The final amount of nananoparticles was filtered (Wattmanpaper 1) to excluded any precipitated and then centrifuged at 14,000 rpm at 4 °C and deep freeze store until used .

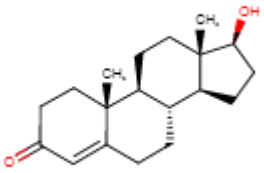
Statistical analysis

The statistical significance of differences among groups (one-way ANOVA method to be statistics[15].

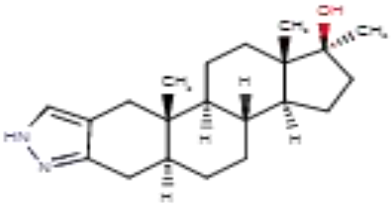
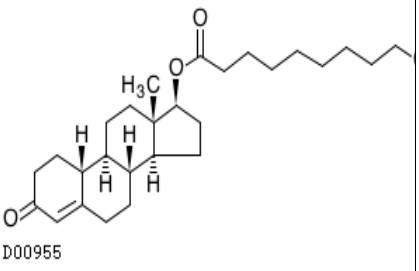
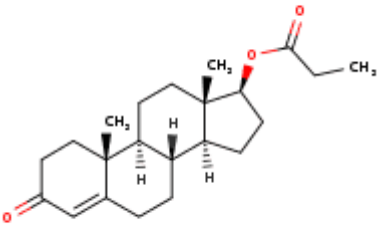
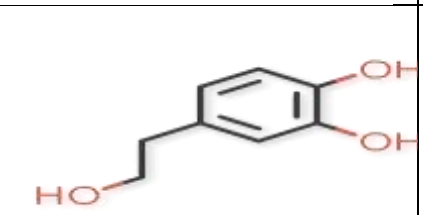
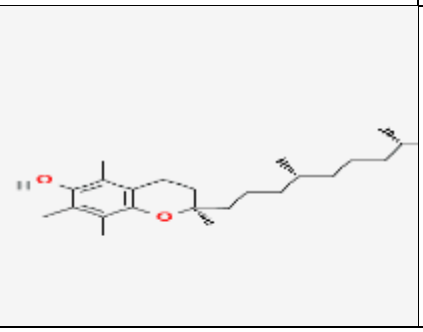
Results

Composition protein of the target The androgenic receptor (AR) (2NW4) was used as the primary treatment target for infertility and hypogonadism. The three dimension composition of androgen receptor was retrieved from Data Bank of Protein with PDB ID: 2NW4.Testosterone, Stanazole, Nandrolonedecanoate, testosterone propionate , Hydroxytyrosol and tocopherol were used. In these top seven assesments , it was found that Hydroxytyrosol Demonstrate the best docking form with binding affinity of –6.2 kcal/mol with AR immediately followed by Stanazole with binding affinity of (−5.2 kcal/mol,figure)(2,3), while tocopherol showed the best docking conformation with binding affinity of (–5.3kcal/mol) with 1D (TNF) as compared with (-5 for kcal/mol) for hydroxytyrosol(Table 1 and figure 3-4).

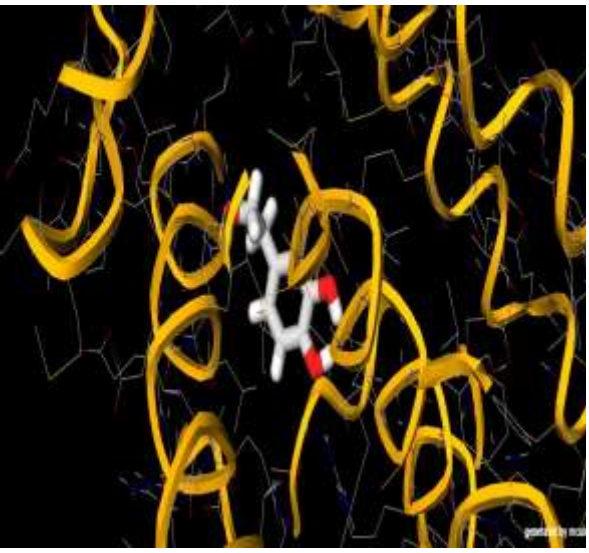
Table (1) Linking affinity energies and catalytic sites in interaction with commercially available fertility medications.

Commercial drugs available as androgenic effect	**structure**	**Binding affinity kcal/mol**	**Target**
Testosterone		**-4.5**	**2NW4 ANDROGEN RECEPTOR)**

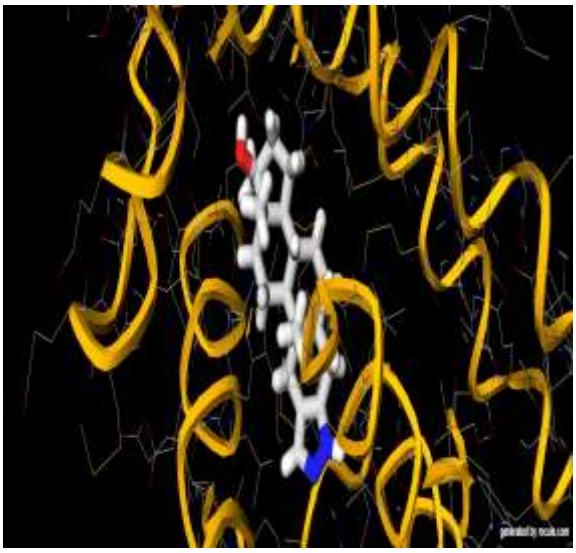
ISSN NO: 2771-8840
Date of Publication: 20-06-2023

Stanazole		**-5.2**	**2NW4 ANDROGEN RECEPTOR)**
Nandrolone decanoate		**-1.3**	**2NW4 ANDROGEN RECEPTOR)**
testosterone propionate		**-1.3**	**2NW4 ANDROGEN RECEPTOR)**
Hydroxytyrosol		**-6.2**	**2NW4 ANDROGEN RECEPTOR)**
Hydroxytyrosol		**5**	**1D00 (TNF RECEPTOR-ASSOCIATED FACTOR 2**
Vitamin E		**5.3**	**1D00 (TNF RECEPTOR-ASSOCIATED FACTOR 2**

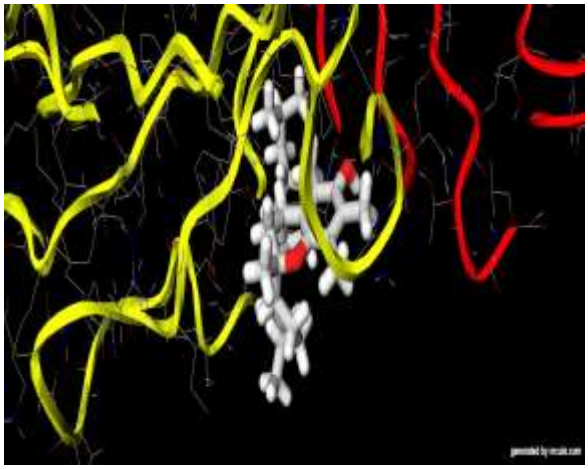
ISSN NO: 2771-8840
Date of Publication: 20-06-2023



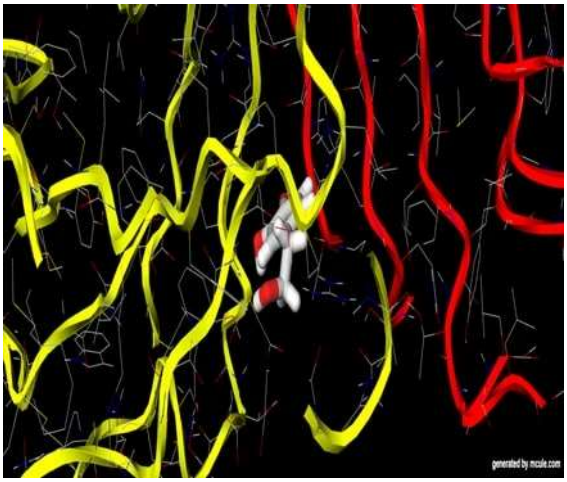
Figure(1): Docking study in AR (2NW4). A Superposed structures of crystallographic conformation of AR (blue) and docked installation (cyan) of HT into AR.



Figure(2): Docking study in AR (2NW4). A Superposed crystallographic conformation structures of the AR (blue) and the docking installation (cyan) of stanazole into the AR.



Figure(3):Docking study in AR (**2NW4**). ASuperimposed structures of crystallographic configuration of AR (blue) and docked pose (cyan) of tocopherol in Tnfa (**1D00**).



Figure(4):Docking study in AR (2NW4). A Superimposed structures of crystallographic configuration of AR (blue) and docked pose (cyan) of HT in Tnfa (1D00).

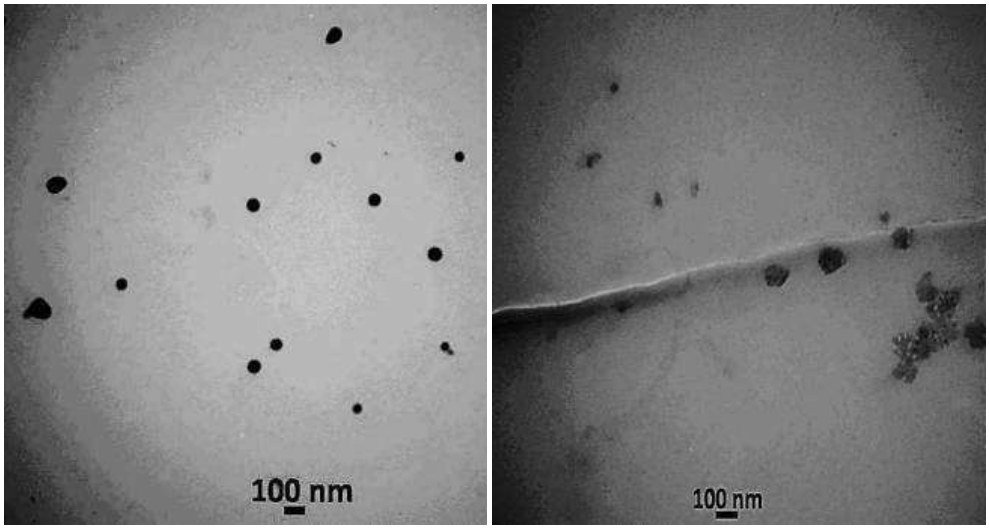
Characterization of hydroxytyrosol nanoparticles:

(Figure 5) and table (2) a typical SEM microscopic picture of chemical and biological SNPs. SNPS was a cubic in shape, uniformly (unified) without a large assembly. The electronic microscopic scan was used to explain with mobility .

ISSN NO: 2771-8840
Date of Publication: 20-06-2023

Table(2) Particle size and polydispersity index (PDI), of Silymarin nanoparticles.

REF.NO	Organic phase	Aqueous phase	Particle size nm	Polydispertion index	Zeta potential
1	Acetone	TPGS	144	0.003	57.3
3	DCM	Tween 80	450	0.37	4.7



Figure(5) Showed TEM image of the particle in size and morphology of the hydroxytyrosolnano

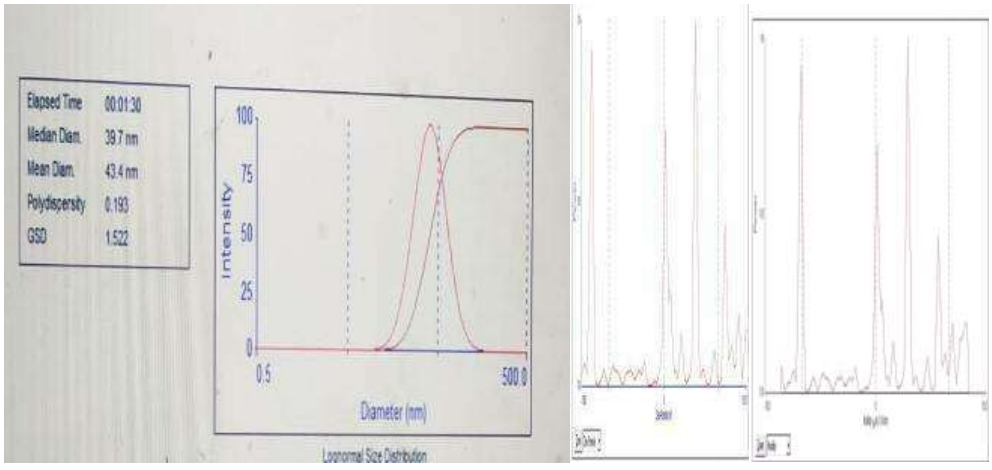


Fig (6) Refer to particle size and polydisperssion index of Hydroxytyrosol nanoparticles and of hydroxttyrosol nanoparticles refer to stable of nanoparticles at 57.3±3.6 (A) and 4.3± 0.28 respectively .

ISSN NO: 2771-8840
Date of Publication: 20-06-2023

SNPS size is measured by light of dynamic of prose. Exposing the size the distribution of nanoparticles parameter method of the chemical method that the average size of the particles was about 39.4 nm.

The effect of hydroxiriosal nanoparticles on the size molecules, and the small molecules that use material via oral nanotechnology produce nanoparticles (NPS) about 39.3 nm. This study and previous studies have applied a different of stabilizing and emulsifying agents, including tocopherol and tween80 .Herein, the nanoparticles of synthesis of fused with emulsifing agent vitamin E (tocopherol) TPGS as with stabilization and reducing of size were reported by numbers of studies. The these study were agreed with multi reports and inhibit accumulation and sedimentation of nanoparticles that create particles due to water immiscible and aggregation due to incompatible with tween 80. Moreover the uptake nanoparticles by cell and limited by large size as well as high polydispersion and low zeta potential under 15 lead to aggregation and accelerate degradation of nanomaterial[16].

Histopathological study

As we expected, May drugs and infection that cause a positive alteration in sperm parameters. In exceptional, we have noticed an increase in the number of sperms, motility and viability in rats received small particles of HT to recorded mean value(50±1.52, 86±3.05505, 10.66±1.45 and 89.66±1.45) for sperm count, sperm viability, Sperm abnormality and motility a significant as compared with control table(2).Administration of HT nanoparticles was capable of improvement at almost all levels of the control groups all the sperm parameters considered as well as that may be also augment serum levels of testosterone was confirmed by[26] noted that olive oil improvement a sperm count and hormones and restore these abnormalities and DNA damage of sperms or at levelof spermatozoa.histopathological section of testes for hydroxytyrosol group showing thickening of seminiferous tubules wall, with decrease in spermatogenesis as well as vacoulation in spermatogonia (black arrow), with increase in spermatogenesis (8-A,B). while clear improvement noted at group received hydroxytyrosol nanoparticles as normal arrangement of spermatogonia (figure 9).

Table(3) : Improvement of sperms parameters of male Rats by consumption nanoparticles of Hydroxytyrosol

TRT	sperm count	sperm viability	Sperm abnormality	motility
Nanoparticles of HT	50±1.52753 A	86±3.05505 A	10.6667±1.45297 B	89.6667±1.45297 A
hydroxytyrosol	44±1.73205 B	82±3.51188 B	13. 33±4.91031 AB	84.6667±1.45297 B
Control	43.6667±2.02759 B	80.3333±2.03 B	14±0.57735 A	86±1.52753 AB
P-value	0.013	0.054	0.166	0.012
*same letter are not significantly different in the same column *Means ± Std. Error				

The histopathological section of the testicles of the nano-hydroxytyrosol group shows the normal disposition of spermatogonia through increased activity and increased absorption by the cell which will reproduce scavenger activity against free radicals. olive oil are benefit because of the majority of HT17.HT has anti-microbial, antioxidant and anti-inflammatory effects which protect the cardiovascular system and account for 40 to 50% of olive oil.The tissue gonads are particularly vulnerable to excessive expression of SAR from unsaturated fatty acids which are dominated by the complex antioxidant system that involves. SODEGSH, and CAT are antioxidant enzymes endogenous type are firstly accountable for removing over production of ROS5, 18, 19., ROS can bind with the unsaturated bonds of lipids membrane leading to an excess cellfree radicals . Oral Administration of HT at the 50 mg/kg dose was of perfect quality for compact lipid peroxidation, as evidenced by the decrease of TBARS20, 21..peroxidation leads to stimulating

ISSN NO: 2771-8840
Date of Publication: 20-06-2023

the paths of internal and external programming cell death signals. , phospholipid has been linked to the specified internal membrane of the mitochondion, cardiipine, peroxide and subsequent products to the formation of fat peroxide by activating the death of the internal programming cells6, 22.showed a significant increase inexpression of Bax and a significant reduction in Bcl-2 as well asthat HT at the dose of 50 mg/kg was suitable of full restoring both expressions at the physiological level.Physiologically the The reproductive system requires ROS for reproduction, but the risk appears to be due to an uncontrolled generation that was corrected by stimulating physiologically ant oxidative systems with natural antioxidant compounds 16, 23.

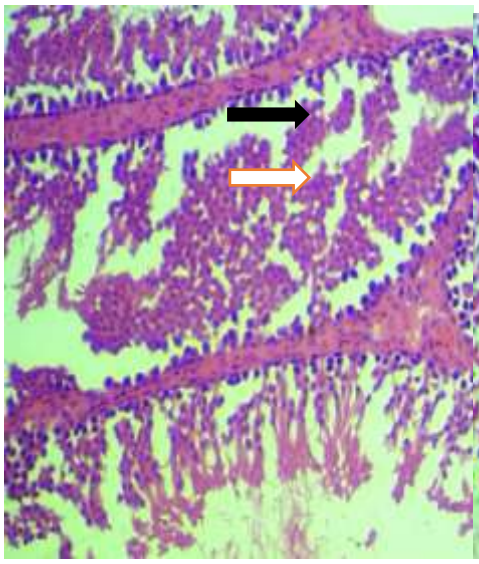


Figure 7 A: histopathological section of testes for hydroxytyrosol group showing thickening of seminiferous tubules wall(black arrow), with marked sloughing of tubular epithelia (white arrow). (H&E stain ,200x)

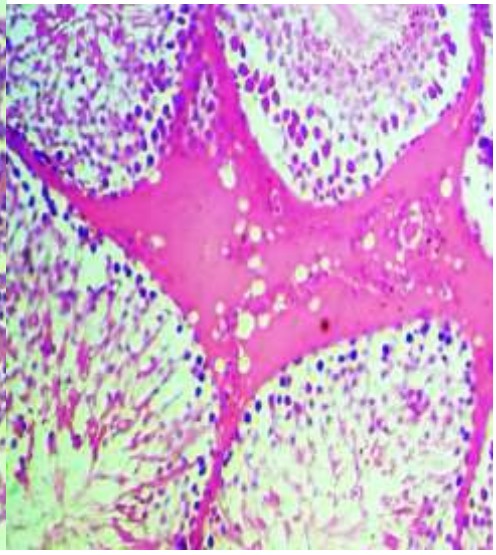


Figure 7 B: histopathological section of testes for hydroxytyrosol group showing severe interstitial edema(black arrow) , with depletion in spermatogonia (white arrow)(H&E stain ,400x)

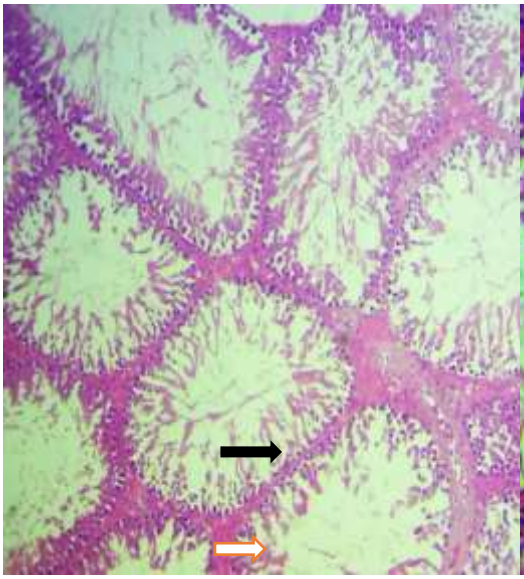


Figure 8: histopathological section of testes for hydroxytyrosol group showing thickening of seminiferous tubules wall(black arrow), with decrease in spermatogenesis (white arrow). (H&E stain ,100x).

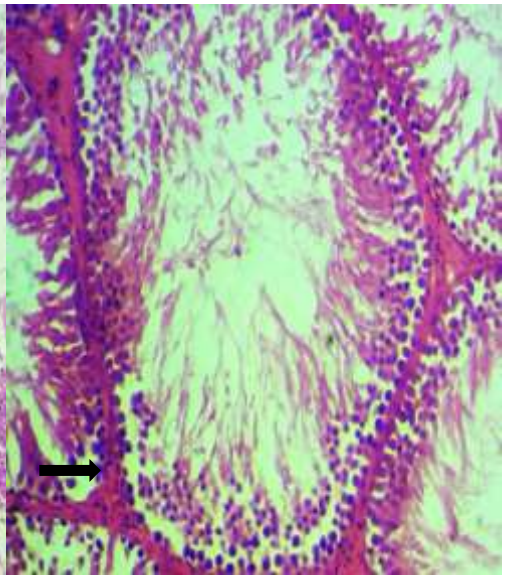


Figure 9histopathological section of testes for nanohydroxytyrosol group showing normal arrangement of spermatogonia (black arrow), (H&E stain ,200x).

ISSN NO: 2771-8840
Date of Publication: 20-06-2023

Conclusion

The present study was confirmed that hydroxytyrosol has potent binding with androgen receptor (2NW4) when compared with other available synthetic compound and confirmed suitable success formulated the compound as nanoparticles that permit good penetration to reproductive tissue and improvement spermatogenesis.

Reference

1. Thongphakdee, A., et al., *Reproductive biology and biotechnologies in wild felids.* Theriogenology, 2020. **150**: p. 360-373.
2. Nishimura, H. and S.W. L'Hernault, *Spermatogenesis.* Current Biology, 2017. **27**(18): p. R988-R994.
3. Shah, W., et al., *The Molecular Mechanism of Sex Hormones on Sertoli Cell Development and Proliferation.* Frontiers in Endocrinology, 2021. **12**.
4. Li, M., et al., *Regulation of spermatogenesis and reproductive capacity by Igf3 in tilapia.* Cellular and Molecular Life Sciences, 2020. **77**(23): p. 4921-4938.
5. Jasim, A.M., H.F. Hasan, and M.J. Awady, *Preparation of Vorapaxar loaded with Vitamin E TPGS and PVA emulsified PLGA nanoparticles In vitro studies.* Research Journal of Pharmacy and Technology, 2019. **12**(9): p. 4503-4510.
6. Jasim, A.M. and M.J. Jawad, *Pharmaceutical Applications of Vitamin E TPGS.* Vitamin E in Health and Disease-Interactions, Diseases and Health Aspects, 2021.
7. Monteiro, M., et al., *Strategies to broaden the applications of olive biophenols oleuropein and hydroxytyrosol in food products.* Antioxidants, 2021. **10**(3): p. 444.
8. Wani, T.A., et al., *Olive oil and its principal bioactive compound: Hydroxytyrosol–A review of the recent literature.* Trends in Food Science & Technology, 2018. **77**: p. 77-90.
9. Zheng, Y., et al., *Metabolism and pharmacological activities of the natural health-benefiting compound diosmin.* Food & Function, 2020. **11**(10): p. 8472-8492.
10. Yuan, J.-J., et al. *Antibacterial activities of polyphenols from olive leaves against Klebsiella pneumoniae*. in *IOP Conference Series: Earth and Environmental Science*. 2021. IOP Publishing.
11. Han, H., et al., *Hydroxytyrosol benefits boar semen quality via improving gut microbiota and blood metabolome.* Frontiers in Nutrition, 2022: p. 1309.
12. Fuloria, S., et al., *A Comprehensive Review on the Therapeutic Potential of Curcuma longa Linn. in Relation to its Major Active Constituent Curcumin.* Frontiers in Pharmacology, 2022. **13**.
13. Al-Zamely, H.A. and Z.S.M. Al-Tamemi, *Role of hydroxytyrosol in ameliorating effects of high fat diet on male rats CNS.* Journal of Pharmaceutical Sciences and Research, 2018. **10**(10): p. 2448-2453.
14. Gaonkar, R.H., et al., *Garcinol loaded vitamin E TPGS emulsified PLGA nanoparticles: preparation, physicochemical characterization, in vitro and in vivo studies.* Scientific reports, 2017. **7**(1): p. 530.
15. Delacre, M., et al., *Taking parametric assumptions seriously: Arguments for the use of Welch's F-test instead of the classical F-test in one-way ANOVA.* International Review of Social Psychology, 2019. **32**(1).
16. Jasim, A.M., et al., *Characterization and Synthesis of Selenium-TPGS Nanoparticles for Target Delivery Clove to Minimize Cytogenic and Liver Damage Induced in Adult Male Rats.* Nano Biomed, 2021. **13**(2): p. 127-136.
17. Dominique, T., et al., *Safety of hydroxytyrosol as a novel food pursuant to Regulation (EC) No 258/97.* 2017.
18. Barati, E., H. Nikzad, and M. Karimian, *Oxidative stress and male infertility: Current knowledge of pathophysiology and role of antioxidant therapy in disease management.* Cellular and Molecular Life Sciences, 2020. **77**(1): p. 93-113.
19. Al-Mansury, S., et al., *Evaluation the Antioxidant Enzymes Activity in Adults Male Rats Treated with Some New 3-mercapto1, 2, 4-triazole Derivatives.* Research Journal of Pharmacy and Technology, 2022. **15**(1): p. 224-228.

ISSN NO: 2771-8840
Date of Publication: 20-06-2023

20. Keçili, R., S. Büyüktiryaki, and C.M. Hussain, *Advancement in bioanalytical science through nanotechnology: past, present and future.* TrAC Trends in Analytical Chemistry, 2019. **110**: p. 259-276.
21. Bader, O.A., et al., *The role of PLGA/TPGS nanoparticle on xylazine-ketamine anesthetic activity in male albino rabbits.* Iraqi Journal of Veterinary Sciences, 2022. **36**(1): p. 201-206.
22. NADIMOHAMED, Nehad, et al. Olive and Basil leave extracts protect against Ibuprofen induced Nephrotoxicity in Albino Rats. *Research Journal of Pharmacy and Technology*, 2020, 13.9: 4190-4194.
23. Kalita, B., Das, M. K., & Sharma, A. K. (2013). Novel phytosome formulations in making herbal extracts more effective. *Research Journal of pharmacy and technology*, *6*(11), 1295-1301.
24. ALAM, Firoj, et al. Naringin: Sources, Chemistry, Toxicity, Pharmacokinetics, Pharmacological Evidences, Molecular Docking and Cell line Study. *Research Journal of Pharmacy and Technology*, 2020, 13.5: 2507-2515. https://doi.org/10.5958/0974-360X.2020.00447.3
25. Purushothaman, B., Suganthi, N., Jothi, A., & Shanmugam, K. (2019). Molecular Docking Studies of potential anticancer agents from Ocimum basilicum L. against human colorectal cancer regulating genes: An insilico approach. *Research Journal of Pharmacy and Technology*, *12*(7), 3423-3427.10.5958/0974-360X.2019.00579.1
26. Othman, S. N. N., Lum, P. T., Sekar, M., Mazlan, N. A., Yusri, P. Z. S., Ghazali, N. F., ... & Noor, A. A. M. (2020). Molecules of interest–embelin–a review. *Research Journal of Pharmacy and Technology*, *13*(7), 3485-3493. https://doi.org/10.5958/0974-360X.2020.00618.6
27. FUSCO, Roberta, et al. Hidrox® counteracts cyclophosphamide-induced male infertility through NRF2 pathways in a mouse model. *Antioxidants*, 2021, 10.5: 778. https://www.mdpi.com/2076-3921/10/5/778.