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Comparative study between the effect of Ginseng extract and Ginseng nanoparticles using SDHA gene expression as an indicator for mitochondrial function in male rats

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Abstract

The current research was conducted to explore the feasibility of employing ginseng nanoparticles (NPs) to improve sperm mitochondrial functions in vivo. For this purpose, a total of (60) adult male Wister rats were explored, acclimated at the animal house of the College of Veterinary Medicine at Al-Qadisiyah University. They prepared and divided into three equal groups. T1, T2, and control negative received only distilled water daily and no other treatment. T1 was given a daily dose of 500 mg/kg of ginseng extract orally by stomach tube. While T2 received orally a daily dose of 250 mg/kg of ginseng NPs by stomach tube. After a 60-day experimental period, all study animals were put to anesthesia and dissection to collect testis samples that were subjected to gene expression (real-time RT, polymerase chain reaction PCR) analysis through targeting Succinate dehydrogenase complex flavoprotein subunit A (SDHA) genes. The results of calculating the gene expression of the gene SDHA showed a significant ($P<0.05$) increase in the level of SDHA gene expression in both the group of rats treated with the Ginseng extract (13.03±1.49) and the group of rats treated with ginseng nanoparticles (48.46±3.85), compared with the negative control group of rats(1±0C). At the same time, the study results showed a significant ($P<0.05$) increase in the level of gene expression in the group of rats treated with nanoparticles when compared with the group of rats treated with extract only. Using sperm analysis and a molecular biologic approach, this study conformal the impact of ginseng on spermatogenesis in rat testes.

Keywords: SDHA gene, testes, nanoparticles (NPs), gene expiration (qPCR), Ginseng.

Introduction

Mitochondria play a function that supportive in the growth of as life evolved from single-celled animals to multicellular ones during the past 1.2–1.5 billion years (1, 2). (3) Claim that mitochondria are intimately connected to a number of essential biological and cellular processes, may be as a result of their role in the relationship between evolution and the fundamental biological circuitry (4). The articles presented have opened up new avenues for diagnosis or therapy using recently found characteristics of mitochondrial biology. Mitochondria affect numerous organ systems systemically. Multiple investigations have shown that a specific alteration of the germ cell's mitochondria is necessary for effective spermatogenesis. In fact, contrary to early spermatocytes and spermatogonia, late spermatocytes, including spermatids, and sperm contain significant quantities of condensed mitochondria (and are hence metabolically more effective). They contain enormous amounts of traditional mitochondria Additionally, some mitochondria are simultaneously losing their function in residual bodies because a significant quantity of cytoplasm is lost during the spermatids' conversion into sperm, a process known as spermiogenesis (5). "Panax ginsengs" is frequently used to describe Asian ginseng. Ginseng has a wide range of physiological effects, including those on the nervous, immunological, and cardiovascular systems. It had been used to boost sexual performance. In countries in East Asia, ginseng It has been put to use for several thousand millenia in the form of conventional medicine to heal ailments. It's become one of the herbs that had been utilized the most frequently during the past 30 years (6). These findings imply that adding ginseng extract to the cryogen used to preserve sperm could increase fertility. Polymeric nanoparticles (PNPs) improved the stability, effectiveness, and security of drug administration both locally and systemically, so they encouraged Increased availability is enabled by effective passage across membrane barriers, while therapeutic site selection is enabled by a change in surface properties (7). To investigate the possibility of use Ginseng nanoparticle to improve sperms mitochondrial functions in vivo.

Materials and Methods:

Animals

Sixty adult male Wister rats weighted 200–250 grams and aged three months old were housed in the animal house at the College of Veterinary Medicine (Al-Qadisiyah University, Al-Diwaniyah, Iraq), and subjected to an acclimation period of 1 week, during which they were kept in a wire-plastic cage under controlled conditions for 12-hour light and 12-hour dark at room temperature and fed on basal laboratory diet tap water. The preparation of ginseng extract and NPs 500g of American ginseng herb was done by taking 100g of the powder of the American ginseng herbal substance and placing it in the thumble of the Soxhlet apparatus in the presence of et70% ethanol, and in this way, American ginseng was extracted. This process is repeated 10–12 times for two days. After completing the process, the material was filtered with filter paper, then placed on a plate and evaporated with the evaporation device for 2–3

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days until the extract was dried. After the fumigation process, the herbal material American ginseng extract was prepared. Then, extract of was dissolved in(PBS) phosphate solution, and the material was placed in the vortexi mixeror five minutes. and then it was placed in the Intellingnt ultrasonic processor using a device of UP200ht at a temperature of 50 ºC for a period of 30 minutes. A solution was obtained. Research was conducted to convert the solutions into nano sizes. After that, it was stored at 4 ºC until use.

Sample collection:

After a week, the research animals were randomly assigned to one of three groups: for study design.

1. First groups (a negative control group): Animals in these groups continue to receive no treatment. and receive simply distilled water every day and 2ml of phosphate buffer solution (PBS).

2. Second groups (T1): Animals in these groups were treated by ginseng extract (500 mg/kg) (2 ml) orally by stomach tube for 60 days.

3. Third groups (T2): Animals in these group were treated by ginseng NPs (250 mg/kg) (2m) orally by stomach tube for 60 days (8).

At the end of an experimental period, All testis tissue samples were collected after all study animals were killed with chloroform.

Molecular examination:

A portion of each research group's testicular tissues were extracted for RNAs according to the manufacturer's instructions for RNA Extraction Kit (Intrron, Korea). In order to detect the SDHA gene [(F:5'-AACACTGGAGGAAGCA CACC-3')]and (R: 5'-GCAACTCGAGTCCCTCACAT-3')] (9), at a product size of 302 bp, Mastermix tubes then created use the {GoTaqTM qPCR}. Mastermix kit (Intrron, Korea) each gene's primers in a final volume of 25 l and subjected to the thermal Cycler [GoTaq® 1-Step RT-qPCR] System settings as follows: 1 reversal of transcription cycle (42C^0 for 15 minutes), 1 cycle RT inactivation/hot-start activation (95 C^0 for 10 minutes), 40 cycles denaturation (95 $C^0$for 10 seconds), annealing/data collection (60 C^0 for 30 seconds) extension (72 C^0 for 30 seconds) as well as one cycle dissociation. The outcomes of the experiments.The fold change test, which is based on the relative assessment of gene expression levels, was used to statistically examine the qRT-PCR experimental data for both the target and housekeeping genes. (10).

Ethical approval:

All techniques involved in the current study were evaluated and authorized by the local Committee for Animal Care and Use at the College of Veterinary Medicine, University of Al-Qadisiyah, Al-Qadisiyah, Iraq.

Statistical Analysis:

All the data are representatives of at least three independents experiments. Values were represented as mean±standard error (M±SE). SAS used a one-way ANOVA to evaluate statistical significance. Differences were considered significant at P<0.05 (*) and P<0.01 (**) (SAS, 2018).

Results:

The results of the molecular study show a considerable variation in the standards of the increase plot of SDHA, the target gene, and the housekeeping gene. (Figure (1)):

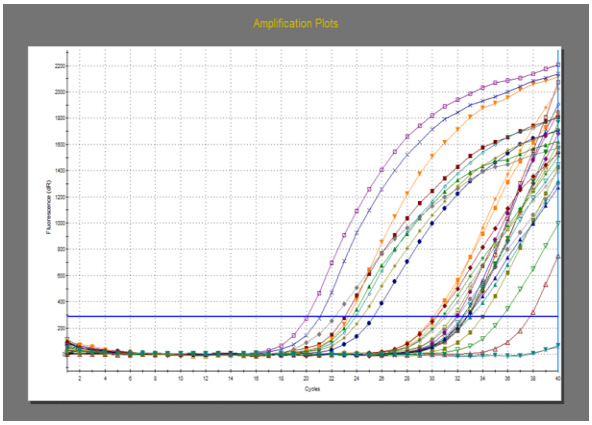

Figure 1: The housekeeping gene (GAPDH) and targets like SDHA were amplified using the Mx3005P

The results of calculating the gene expression of the gene SDHA indicated an significant (P<0.05) increase in the level of genetic material expression in both the group of rats treated with the ginseng extract and the groups of rats treated with ginseng nanoparticles , compared with the negative control group . At the same time, the study results indicated asignificant (P<0.05) increase in the level of genetic material expression in the group of rats treated with nanoparticles when compared with the group of rats treated with extract only. (Table 1)

Table 1: Fold change of the SDHA gene in Ginseng nanoparticles and other groups .

Groups	Fold change (mean ± SE)
Control	1±0C
Ginseng Extract	13.03±1.49B
Ginseng Nanoparticles	48.46±3.85A
P value	<0.0001
LSD(P<0.05)	8.26

Different letters between any two means denote to the significant difference at P<0.05

Discussion:

Studies have demonstrated that ginseng protects mice against testicular toxicity induced by doxorubicin (11). These findings emphasize the importance of (SDHA) and mitochondrial role in sperm health.

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Successful reproduction requires sperm motility. We demonstrate that the mitochondrial enzyme SDHA-2 is necessary affects sperm activation and motility, and thus male fertility motility. SDHA has been discovered to play a new role in sperm activation and motility in this study. Male sperm activate in different ways, with different genetic prerequisites (12). However, only few studies have looked at how ginseng affects male reproductive processes using rat testis. Using sperm analysis and a molecular biologic approach, this study examined the impact of ginseng on spermatogenesis in rat testis. NPs have multiple applications, such as fluorescence imaging in the reproductive system, drug delivery, and medication administration. One significant method of absorption for NPs is ingestion and exposure to the gastrointestinal tract (GIT). NPs have the ability to enter the bloodstream through the GIT and be absorbed, making it simple for them to accumulate in the secondary organs (13).Nanoparticles(NPs) indirectly enter the body through ingestion as humans take food additives, ingredients, and supplements that contain different types of nano NP (14). Allows nanoparticles to circulate, where they are then transferred to numerous body tissues and organs before aggregating in the reproductive organs. Allows nanoparticles to circulate, where they are then transferred to numerous body tissues and organs before aggregating in the reproductive organs(15). Because of their nanoparticles size, NPs can cross biological barriers such as the blood-testis barrier (BTB) (16,17). This protects the reproductive tissues (18). Nanoparticales markers improve optical contrast and molecular specificity in reproductive organelle imaging and aid in the detection of cell organelles and suborganelles. Solution biomarkers are a type of nanotechnology that can attach to numerous sick cells inside the reproductive system, allowing for analysis and treatment (19). Nanotechnology has enabled increased nanoparticle (NP) exposure to the male reproductive system. Since then, assisted reproductive technology revolutionized the treatment of infertility in men but this method has generated questions the dangers that are taken, such as genetic anomalies, structure deformities, and issues due to the potential mechanical and biochemical harm (20; 21; 22). This finding has increased the need for more research to evaluate potential over expression and provide more details regarding mitochondrial protein function in the spermatogenic process. The proteins connected to the mitochondria's respiratory chain, SDHA, and the first enzyme in the Krebs cycle that catalyzes the synthesis of citrate from oxalacetate and acetyl CoA are used to identify mitochondrial malfunction (23). The current work adds to our understanding of Panax quinquefolium's capacity to improve male copulatory behavior in rats. Although one previous study found that Panax. ginseng could enhance male rat copulatory behavior (24), Early research found that prolonged treatment with Panax ginseng resulted in a dose-related increase in male rats' blood testosterone levels(25). Ginseng has been demonstrated to have Penile tissue directly affected, which may contribute to its copulatory performance-enhancing effects. It was recently discovered that P. ginseng extract increased nitric oxide release from endothelial cells in the rabbit penile corpus cavernosum and promoted corpus cavernosum relaxation (26).

Conclusion

Ginseng extract and nanoparticles cause an increase in Succinate dyhaydroginase complex flavoprotain subunit A (SDHA) expression, which means improvement of mitochondrial function and then sperm activity, with an advantage to nanoparticles as compared with extract.

Conflict of interest

The current study has no conflict of interest as declared by the authors

References

1. Wallace DC. Bioenergetics, the origins of complexity, and the ascent of man. Proc Natl Acad Sci U S A. 2010;107(Suppl 2):8947-53. https://doi.org/10.1073/pnas.0914635107

2. Lane N, Martin W. The energetics of genome complexity. Nature. 2010;467(7318):929-34. https://doi.org/10.1038/nature09486

3. Nunnari J, Suomalainen A. Mitochondria: in sickness and in health. Cell. 2012;148(6):1145-59. https://doi.org/10.1016/j.cell.2012.02.035

4. Chandel NS. Evolution of mitochondria as signaling organelles. Cell Metab. 2015;22(2):204-6. https://doi.org/10.1016/j.cmet.2015.05.013

5. Park YJ, Pang MG. Mitochondrial functionality in male fertility: from spermatogenesis to fertilization. Antioxidants. 2021;10(1):98. https://doi.org/10.3390/antiox10010098

6. Yu Y, Jia T, Chen X. The 'how' and 'where' of plant microRNAs. New Phytol. 2017;216(4):1002-17. https://doi.org/10.1111/nph.14834

7. Aldulemy QLM, Abdul-Razak MMA. Effect of Nano Seaweed Extract on Tillaring Pattern, Growth, and Yield of Barley Varieties. Int J Aquat Sci. 2021;12(2):5385-400.

8. Kamel H, Longstreth WT Jr, Tirschwell DL, Kronmal RA, Broderick JP, Palesch YY, et al. The AtRial Cardiopathy and Antithrombotic Drugs In prevention After cryptogenic stroke randomized trial: Rationale and methods. Int J Stroke. 2019;14(2):207-14.

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www.qu.edu.iq/journalvm

https://doi.org/10.1177/1747493018799981

9. Piantadosi CA, Suliman HB. Transcriptional regulation of SDH a flavor protein by nuclear respiratory factor-1 prevents pseudo-hypoxia in aerobic cardiac cells. J Biol Chem. 2008;283(16):10967-77.
https://doi.org/10.1074/jbc.M709741200

10. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods. 2001;25(4):402-8.
https://doi.org/10.1006/meth.2001.1262

11. Kang IS, Jin K, Wang B, Lau KM, Shukla J, Krishnamurthy V, et al. Intercomparison of the climatological variations of Asian summer monsoon precipitation simulated by 10 GCMs. Clim Dyn. 2002;19:383-95.
https://doi.org/10.1007/s00382-002-0245-9

12. Ellis RE, Stanfield GM. The regulation of spermatogenesis and sperm function in nematodes. Semin Cell Dev Biol. 2014;29:17-30.
https://doi.org/10.1016/j.semcdb.2014.04.005

13. Hansson GC. Role of mucus layers in gut infection and inflammation. Curr Opin Microbiol. 2012;15(1):57-62.
https://doi.org/10.1016/j.mib.2011.11.002

14. Bergin IL, Witzmann FA. Nanoparticle toxicity by the gastrointestinal route: evidence and knowledge gaps. Int J Biomed Nanosci Nanotechnol. 2013;3(1-2):163-210.
https://doi.org/10.1504/IJBNN.2013.054515

15. Wang Y, Qiu G, Wang R, Huang S, Wang Q, Liu Y, et al. Field-effect transistors made from solution-grown two-dimensional toluene. Nat Electron. 2018;1(4):228-36.
https://doi.org/10.1038/s41928-018-0058-4

16. Lan Z, Yang WX. Nanoparticles and spermatogenesis: how do nanoparticles affect spermatogenesis and penetrate the blood-testis barrier. Nanomedicine. 2012;7(4):579-96.
https://doi.org/10.2217/nnm.12.20

17. Saeed A, Abdulwahid M. Investigation of the effects of green synthesized ZnO nanoparticles on the viability of L929 fibroblasts using MTT assay. QJVMS. 2023;22(1):119-25.

18. Rollerova E, Tulinska J, Liskova A, Kuricova M, Kovriznych J, Mlynarcikova A, et al. Titanium dioxide nanoparticles: some aspects of toxicity/focus on the development. Endocr Regul. 2015;49(2):97-112.
https://doi.org/10.4149/endo_2015_02_97

19. Horne AW, Duncan WC, Critchley HO. The need for serum biomarker development for diagnosing and excluding tubal ectopic pregnancy. Acta Obstet Gynecol Scand. 2010;89(3):299-301.
https://doi.org/10.3109/00016340903568191

20. Hindryckx P, De Vos M, Jacques P, Ferdinande L, Peeters H, Olievier K, et al. Hydroxylase inhibition abrogates TNF-α-induced intestinal epithelial damage by hypoxia-inducible factor-1-dependent repression of FADD. J Immunol. 2010;185(10):6306-16.
https://doi.org/10.4049/jimmunol.1002541

21. Glaser J, Nguyen TD, Anderson JA, Lui P, Spiga F, Millan JA, et al. Strong scaling of general-purpose molecular dynamics simulations on GPUs. Comput Phys Commun. 2015;192:97-107.
https://doi.org/10.1016/j.cpc.2015.02.028

22. Ribas-Maynou J, Yeste M. Oxidative stress in male infertility: causes, effects in assisted reproductive techniques, and protective support of antioxidants. Biol. 2020;9(4):77.
https://doi.org/10.3390/biology9040077

23. Lee K, Malerba F. Catch-up cycles and changes in industrial leadership: Windows of opportunity and responses of firms and countries in the evolution of sectoral systems. Res Policy. 2017;46(2):338-51.
https://doi.org/10.1016/j.respol.2016.09.006

24. Frederickson GN, Hecht MS, Kim CE. Approximation algorithms for some routing problems. In: 17th annual symposium on foundations of computer science (sfcs 1976); 1976 Oct; IEEE. p. 216-27.
https://doi.org/10.1109/SFCS.1976.6

25. Fahim MS, Fahim Z, Harman JM, Clevenger TE, Mullins W, Hafez ESE. Effect of Panax ginseng on testosterone level and prostate in male rats. Arch Androl. 1982;8(4):261-3.
https://doi.org/10.3109/01485018208990207

26. Chen X, Lee TJ. Ginsenosides-induced nitric oxide-mediated relaxation of the rabbit corpus cavernosum. Br J Pharmacol. 1995;115(1):15.
https://doi.org/10.1111/j.1476-5381.1995.tb16313.x