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Mechanistic Insights into Ginger's Role in Improving Sperm Malformation Under Heat Stress Using Network Pharmacology and Molecular Docking

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Abstract: *Objective:* This study investigates the effects of ginger on sperm dysmorphosis under heat stress using network pharmacology and molecular docking techniques. *Methods:* The core components, target sites, and primary pathways of ginger were predicted using the TCMSP database. Genes associated with sperm malformation and heat stress were identified through databases such as GeneCards and DisGeNET, followed by KEGG and GO analyses based on intersections with ginger targets. Core genes identified via Cytoscape software were subjected to molecular docking with ginger's active components. *Results:* The principal active components of ginger identified include β-sitosterol and dihydrocapsaicin. Network pharmacology analysis suggested that ginger exerts its effects through pathways such as the prostate cancer signaling pathway, estrogen signaling pathway, inflammatory pathways, and nuclear receptor signal transduction. These pathways may ameliorate sperm malformation symptoms caused by heat stress. *Conclusion:* Integrating findings from network pharmacology and molecular docking, the active components of ginger potentially modulate the expression of heat shock proteins during heat stress via inflammatory and oxidative stress pathways. This modulation may protect spermatogenesis under heat stress and improve sperm malformation.

Keywords: Network pharmacology; Molecular docking; Ginger; Heat stress; Heat shock protein

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1. Introduction

Ginger (*Zingiber officinale* Roscoe) is a traditional Chinese medicinal material widely utilized for both culinary and therapeutic purposes. It contains volatile oils, gingerol, linear diphenylheptanes, ginger polysaccharides, trace

elements, and various other chemical constituents. Modern pharmacological studies have demonstrated that ginger exhibits antibacterial, anti-inflammatory, analgesic, antioxidant, and anti-tumor properties [1]. Initially documented in the "Synopsis of Prescriptions of the Golden Chamber," ginger is described as mild in nature and tangy in taste, offering benefits such as warming the middle, dispelling cold, removing dampness, and alleviating respiratory and digestive issues.

Elevated environmental temperatures leading to increased testicular temperature can adversely affect spermatogenesis, ultimately reducing male fertility or causing sterility [3]. Literature suggests that heat stress decreases sperm count, disrupts the integrity of the blood-testis barrier [4], and induces oxidative stress in testicular germ cells, resulting in apoptosis and DNA damage [5]. The regulatory mechanisms underlying the negative effects of heat stress on spermatogenesis remain unclear. Further research is urgently needed to elucidate the mechanisms of oxidative stress-induced DNA damage and sperm malformation.

Ginger polysaccharides, polyphenols, and extracts have demonstrated significant antioxidant capabilities [6-8]. Using network pharmacology and molecular docking techniques, this study systematically analyzed the effective chemical constituents of ginger, along with associated target genes and signaling pathways, in treating oligospermia and sperm malformation. This approach aimed to elucidate the mechanisms through which ginger alleviates sperm malformation, providing both theoretical and practical insights into its role in mitigating germ cell disorders caused by heat stress.

2. Data and methods

The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), PubChem (https://pubchem.ncbi.nlm.nih.gov/), and SwissTargetPrediction were utilized to retrieve and predict the targets of the primary components of ginger. The identified targets were translated using the UniProt online platform, where the corresponding gene IDs were obtained. GeneCards (https://www.genecards.org/) and DisGeNET (https://disgenet.cn/) were used to identify disease-related gene targets associated with the manifestations of spermatic dysmorphia.

Disease gene targets were determined using the keywords "teratozoospermia" and "oligospermia," while heat stress-related genes were identified with the keyword "Heat Stress Disorders." After correction and de-duplication, overlapping targets were identified through Venny 2.1.0. The common targets obtained from the intersection were imported into the STRING database to construct a protein-protein interaction (PPI) network.

R software was employed for Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the core gene targets. Molecular docking was performed between the main active components of ginger and the core targets using LeDock V1.0 software, and the results were visualized using PyMOL 4.3 software.

3. Results

3.1. Network pharmacology results

Using the GeneCards and DisGeNET databases, 8,923 heat stress-related genes were identified with the keyword "Heat Stress." In conjunction with the manifestations of sperm malformation, terms such as "teratozoospermia" and "oligospermia" were utilized to identify 1,694 disease-related gene targets. Additionally, through TCMSP,

PubChem, and SwissTargetPrediction, 131 targets were predicted and integrated for the main active components of ginger. Following integration and de-duplication, 20 intersection targets were identified using Venny 2.1.0 (**Figure 1A**). These targets were subsequently imported into the STRING database to construct a PPI network, and Cytoscape 3.8.0 software was used to generate the component-target relationship diagram (**Figure 1B**).

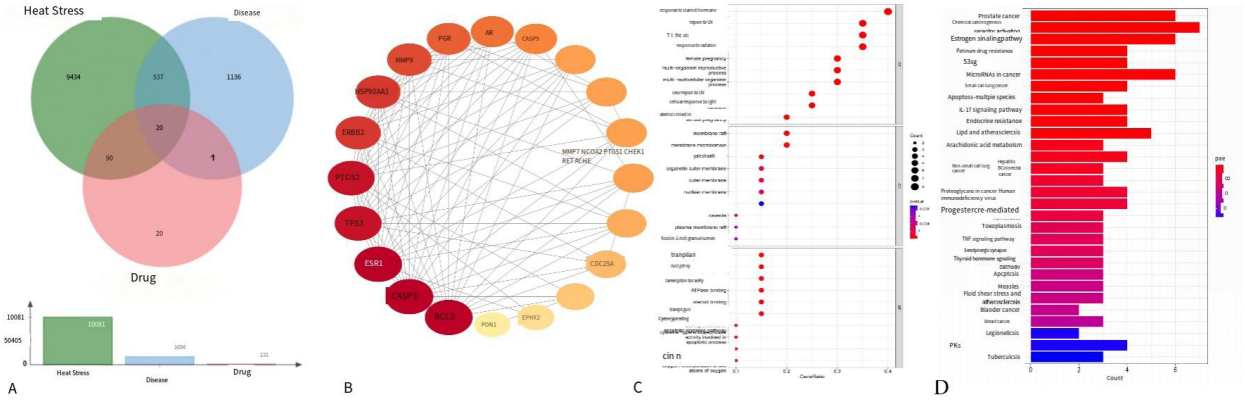

Figure 1. (A) Intersection target Wayne Diagram; **(B)** Target protein interaction diagram; **(C)** GO diagram; **(D)** KEGG analysis

GO enrichment analysis (**Figure 1C**) and KEGG pathway analysis (**Figure 1D**) were conducted for the intersection genes identified in **Figure 1A**. In the biological processes (BP) category, genes were primarily enriched in pathways related to the response to light stimulus, UV radiation, radiation, and steroid hormone signaling. In the cellular components (CC) category, the results predominantly involved various biofilm-related functions. Within the molecular functions (MF) category, genes were enriched in pathways regulating transcription and redox enzyme activity. KEGG analysis revealed that the genes were significantly enriched in pathways such as the prostate cancer pathway, estrogen signaling pathway, p53 signaling pathway, IL-17 signaling pathway, and NF-κB signaling pathway.

3.2. Molecular docking results

The PPI network was further analyzed using Cytoscape 3.8.0 software. Core targets, including HSP90AA1, ERBB2, ESR1, and PGR, were identified based on node scores calculated through the CytoNCA plugin. Molecular docking was conducted using LeDock to evaluate interactions between the primary active components of ginger and these core targets.

The docking results demonstrated that compounds such as β-sitosterol and dihydrocapsaicin formed stable interactions with the core targets (e.g., HSP90AA1, ERBB2, ESR1, and PGR) through hydrogen bonds and hydrophobic interactions. Binding energies were ≤ -5.0 kcal/mol, indicating strong binding affinities. Partial visualizations of these interactions are displayed in **Figure 2**.

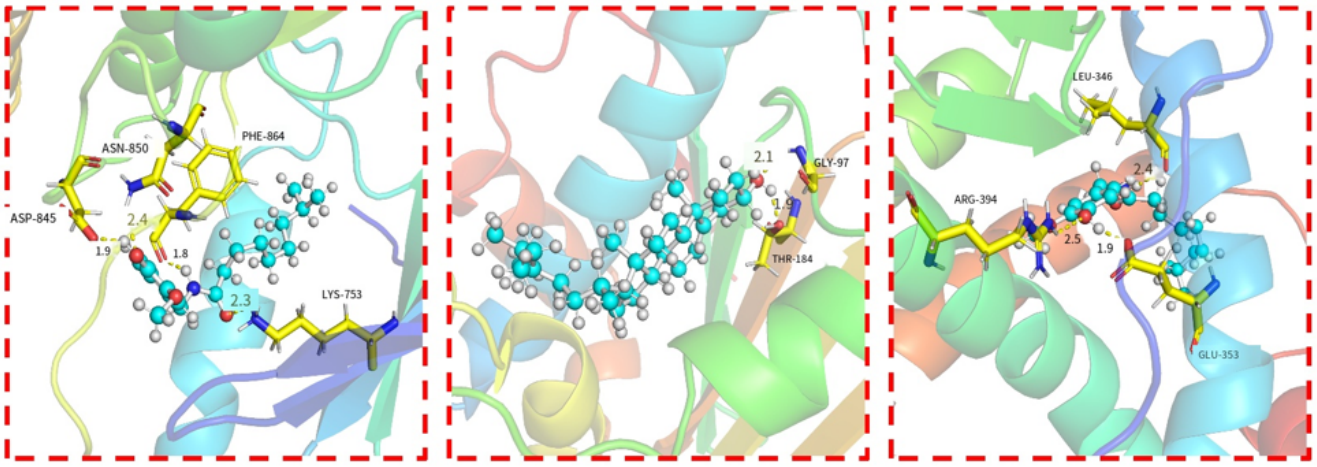

Figure 2. (Left) The 3D diagram of β-sitosterol docking with ERBB2; **(Middle)** The 3D diagram of dihydrocapsaicin docking with HSP90AA1; **(Right)** The 3D diagram of dihydrocapsaicin docking with ESR1

4. Discussion

GO enrichment analysis of the intersection genes revealed significant enrichment in pathways related to the response to light stimulus, UV, and radiation, all of which are closely associated with heat stress. UV exposure can induce DNA damage, potentially leading to chromosomal alterations in sperm, which in turn may contribute to sperm malformation [5]. Furthermore, the enrichment of genes in pathways regulating redox enzyme activity suggests their potential involvement in the redox reactions within the body. This finding aligns with previous research indicating that ginger possesses potent anti-inflammatory and antioxidant properties [6-8].

The KEGG enrichment analysis indicated that the intersection genes were significantly enriched in pathways such as the prostate cancer signaling pathway, estrogen signaling pathway, p53 signaling pathway, IL-17 signaling pathway, NF-κB signaling pathway, as well as apoptosis and necrosis pathways. Among these, the HSP90AA1 protein, a heat shock protein whose expression increases under elevated temperatures, plays a role in antioxidant and anti-apoptotic mechanisms, which are hallmarks of heat stress. Both HSP90AA1 and ERBB2 were identified as key targets within the prostate cancer pathway, suggesting a potential link between high temperatures and prostate cancer development.

The estrogen signaling pathway, involving genes such as PGR, HSP90AA1, and ESR1, is associated with inflammation, while the NF-κB signaling pathway is directly involved in the body's inflammatory response. The molecular docking results demonstrated that the active components of ginger, such as gingerol and dihydrocapsaicin, might influence the biological activity of target proteins by altering the conformation of their active centers. It is hypothesized that these active components exert anti-inflammatory and antioxidant effects through the aforementioned pathways, thereby alleviating sperm malformation symptoms caused by heat stress.

The testicular tissue within the male reproductive system is particularly sensitive to temperature changes, making the impact of heat stress on male fertility a critical area of research [9]. Heat stress is known to induce oxidative stress in spermatocytes, leading to the elevated expression of heat shock proteins, which may in turn trigger endoplasmic reticulum stress (ERS). This cascade can result in apoptosis of spermatocytes, ultimately reducing spermatogenesis. Oxidative stress and DNA damage are significant contributors to sperm malformation

[5,10]. Additionally, DNA damage incurred during heat stress may lead to genetic mutations, altering germline genetic material and causing deformations in sperm [11-15].

The antioxidant compound gingerol, present in ginger, is recognized as an inhibitor of prostaglandin synthase. It exhibits multifaceted anti-inflammatory and antioxidant functions, mitigating the damage caused by reactive oxygen species generated during heat stress. Gingerol reduces the oxidative stress-induced damage to spermatocyte DNA, alleviates ERS, lowers spermatocyte apoptosis, and helps maintain normal spermatogenesis. These mechanisms collectively contribute to the improvement of symptoms associated with sperm malformation under heat stress.

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Disclosure statement

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