Screening the possible effect of a phytofabricated nanoselenium-composite from *Eruca sativa* extract in reducing infertility in males

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The antifertility effects of ketoconazole can be avoided or diminished by administering nano-selenium-based-antioxidant plant extract simultaneously or sequentially. Using selenium as nanoparticles (SeNPs) is one of the essential methods for enhancing its therapeutic effects and lowering toxicities. This study therefore aimed to analyze the changes made to the parameters of androgens, such as testosterone and gonadotropin hormones: luteinizing hormone and follicle stimulating hormones together with sperm indexes after administration of antioxidant phytofabricated nanoselenium to mitigate oxidative damage brought on by ketoconazole. In brief, 1% weight-per-volume of the extract was loaded into a solution of 10 mM sodium selenite in various ratios on a magnetic stirrer (50 °C, PH 9) in the dark for 12 hours, left for 48 hours and then sent for characterization, which was performed using ultraviolet-visible spectroscopy (UV-vis spectra), Fourier transform infrared spectroscopy (FTIR), and dynamic light scattering (DLS). Then after the selection of the solution containing the optimal fabricated selenium nanoparticles it was administered to three groups out of seven groups of albino rats with eight animals in each one as follows: Gr. A negative control (no treatment), Gr. B oral ketoconazole 50 mg/kg for fourteen days, Gr. BC, BD, BE1, BE2 and BE3; each one received oral ketoconazole 50 mg/kg for fourteen days followed by: 200 mg/kg *Eruca sativa* (Gr. BC), 0.5 mg/kg oral sodium selenite (Gr. BD), 0.5 mg/kg/cm² skin area local nanoselenium (Gr. BE1), 0.25 mg/kg oral nanoselenium (Gr. BE2) and 0.5 mg/kg oral nano selenium (Gr. BE3) respectively for 28 days. After this period, the animals were anesthetized, and plasma testosterone, luteinizing, and follicle stimulating hormones were assessed using Elisa Kit, after that, they were euthanized, and the epididymis of the right testis was carefully removed for evaluation of sperm indices (count, viability, abnormality, and motility). The reduction of selenium ions into PF-SeNPs induced by *Eruca sativa* extracts at a ratio of (1:2) (Na-SeO₄: Eruca sativa) solution was confirmed by the gradual conversion of colour from dark brown to light yellow and then to reddish-orange after the addition of acidic sodium selenite solution and reacting for 12 h. The final reddish-orange colour is the most significant property of nanoparticles. In UV-vis spectroscopy, a strong absorption peak appeared between 268-364 nm with maxima at 268 nm, confirming the formation of nanoselenium. The optimal phytofabricated nanoselenium particles were obtained with a spherical shape, highly stable, and the smallest in size (39-44 nm in diameter) as proved by DLS with Poly Dispersion Index of 0.242 and zeta potential value of ~66.57 mV. The measurement of inhibition of testicular damage was linked to significant reductions in testosterone levels, elevated levels of LH and FSH, and significant decreases in sperm count, motility, and viability, which in turn affected spermatogenesis. Concurrently, the administration of *Eruca sativa* extract, sodium selenite and nanoselenium solution (in different doses and routes) following ketoconazole was shown to significantly improve biochemical parameters. These improvements included an increase in testosterone levels with little to no impact on LH and FSH levels as well as improved sperm indices. Additionally, the oral nanoselenium groups in 0.25 and 0.50 mg/kg produced the best outcomes with only minor differences between them. In conclusion, the antioxidant effects of the phytofabricated selenium-based *Eruca sativa* leaf extract considerably improved testicular tissues.

Keywords: selenium; nanoselenium; testosterone; luteinizing hormone; follicle-stimulating hormone; sperm indices.

Introduction

Selenium is integrated in selenoproteins that are high in selenocysteine and is one of the most important trace elements in a number of important metabolic processes. It serves as a cofactor for several enzymes that perform oxidoreductase functions, including glutathione peroxidase and thioredoxin reductase (Aslani, 2017). These enzymes have cytoprotective properties, and their activation promotes fertility, lessens inflammatory responses, and acts as a chemo preventive against many malignancies (Landis-Piwowar & Iyer, 2014). In this regard, free radical-induced oxidative stress is thought to be the most significant risk factor that threatens the testicular function. Therefore, an antioxidant defense system, represented by enzymes included in selenoproteins, can combat these free radicals or prevent their formation in the testicular cells (Assairi et al., 2020; Salman et al., 2022). An innovative, expanding field, nanotechnology has significant uses in both science and technology. Nanoparticles are used as a platform for carriers in the pharmaceutical industry to transport medications to their sites of action. It is based on the production of tiny (less than 100 nm) sized particles (Khorana et al., 2019), which improve the biological activity, reduce toxicity, and permit the controlled release of medicines, particularly in capsule form (Patra et al., 2018). By using chemical or biological reducing agents, metal nanoparticles can be created. At the same time, scientists discovered that utilizing plant extracts with strong antioxidant properties may assist in accomplishing that goal. As the nanoparticle formulation will boost the penetration of selenium and the plant extract into the target region, this may help create a synergistic impact, allowing such formulations to kill two birds with one stone (Khameneh, 2021). The antifungal medication ketoconazole has been linked to decreased serum testosterone levels, decreased weight of male
reproductive organs, especially the testes, and decreased epididymis sperm concentration. These changes were examined by measuring the levels of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) in plasma samples obtained from the rats treated with Se NPs. The animals were euthanized and the testes were excised and weighed. The serum samples were analyzed for hormone levels using ELISA, and the results were statistically analyzed using ANOVA with Bonferroni correction. The results showed a significant increase in LH levels in the treated groups compared to the control groups. The effect of the different Se NP concentrations on the reproductive system was also assessed by examining the sperm parameters and histological sections of the testes. Overall, the results indicated that the synthesized Se NPs had a positive effect on the reproductive system of male rats, with the highest effect observed in the group treated with the highest Se NP concentration.
Fig. 1. FTIR spectra of *Eruca sativa* extract, sodium selenite solution, and phytofabricated selenium nanoparticles (PF-SeNPs) (Shareef et al., 2023)

### Table 2
Luteinizing hormone for the different control and treated groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>LH in mIU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr. A</td>
<td>3.92 ± 0.22</td>
</tr>
<tr>
<td>Gr. B</td>
<td>5.70 ± 0.28*</td>
</tr>
<tr>
<td>Gr. BC</td>
<td>4.98 ± 0.24</td>
</tr>
<tr>
<td>Gr. BD</td>
<td>5.50 ± 0.28*</td>
</tr>
<tr>
<td>Gr. BE1</td>
<td>5.12 ± 0.26</td>
</tr>
<tr>
<td>Gr. BE2</td>
<td>5.34 ± 0.22*</td>
</tr>
<tr>
<td>Gr. BE3</td>
<td>4.86 ± 0.42</td>
</tr>
</tbody>
</table>

*Note*: see Table 1.

Follicle-stimulating hormone (mIU/mL) for the different control and treated groups: normal FSH level in Gr. A control group ranged between (4.011–4.943 mIU/mL). Results showed a significant increase in FSH among Gr. B, BD, and BE3 compared to Gr. A (Table 3).

### Table 3
Follicle stimulating hormone (mIU/mL) for the different control and treated groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>FSH mIU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr. A</td>
<td>4.48 ± 0.21</td>
</tr>
<tr>
<td>Gr. B</td>
<td>5.51 ± 0.16*</td>
</tr>
<tr>
<td>Gr. BC</td>
<td>5.20 ± 0.16</td>
</tr>
<tr>
<td>Gr. BD</td>
<td>5.58 ± 0.19*</td>
</tr>
<tr>
<td>Gr. BE1</td>
<td>5.29 ± 0.24</td>
</tr>
<tr>
<td>Gr. BE2</td>
<td>5.14 ± 0.26</td>
</tr>
<tr>
<td>Gr. BE3</td>
<td>5.55 ± 0.26*</td>
</tr>
</tbody>
</table>

*Note*: see Table 1.

**Sperm count.** Groups B, BC, BD, and BE1 showed a highly significant decrease in sperm count compared to Group A. At the same time, Group BE2 showed a significant decrease compared to Gr. A, but a highly significant increase in the count in comparison to Gr. B and a significant increase compared to groups BC and BD. At the same time group BE3 exhibited a highly significant increase in sperm count compared to groups B, BC, and BD with non-significant differences with groups A, BE1, and BE2 (Fig. 2).

**Sperm viability and abnormalities.** Groups B and BC exhibited the highest significant values of dead sperm compared to Gr. A, also there was a significant decrease in the number of dead sperm among groups BC, BD, BE1, BE2, and BE3 in comparison with group B but this did not
reach the control values. Groups B, BC, and BD showed a significant increase in abnormal sperm compared to group A. Groups BC, BD, BE1, BE2, and BE3 showed a significant decrease in the number of abnormal sperm and an increase in normal cells compared to Group B. At the same time, Group BE1 showed a significant increase in normal cells and decrease in abnormal ones compared to Gr. BC, while both groups BE2 and BE3 exhibited a significant increase in the normal cells and a decrease in the abnormal cells compared to Group BD (Fig. 3).

Discussion

Testosterone (T) secretion and the gonadotropic hormones LH and FSH are required for normal spermatogenesis (Odnowe et al., 2018). FSH acts directly on the seminiferous tubules, whereas LH indirectly increases steroidogenesis by causing the mitochondrial conversion of cholesterol to pregnenolone and testosterone in Leydig cells. Therefore, a drop in FSH worsens testicular damage, but a rise in T level indicates how much spermatogenesis has been altered and how much spermatogenic cell depletion has occurred (Smith & Walker, 2014).

In the present study, KET-induced reproductive system toxicities, including a decrease in serum T level with increases in both LH & FSH levels (Amin, 2008), indicated testicular damage resulting in hypergonadism. There are numerous causes for this hazardous impact, including: the enzyme C17,20 lyase is inhibited by KET, preventing it from converting 17α-hydroxyprogesterone to androstenedione (Alizadeh et al., 2018). Another method by which KET-induced testicular toxicity was caused was ROS production and oxidative damage to lipid membranes (Durbandi et al., 2018).

All PF-SeNPs groups (BE1, BE2 and BE3) retained normal testosterone levels in a dose-dependent manner, especially BE3, which showed the highest T value in contrast to the other treatment groups, which showed significant increment in testosterone level relative to the B group but did not retain normal hormone level as in Group A. These effects, which are probably brought on by PF-SeNPs antioxidant activity and defense of the testicular tissue against oxidative stress and damage, are anticipated to enhance T levels and overall gonadal function (El-Kazz et al., 2020). Following KET administration for fourteen days, groups BC, BD, BE1, BE2, and BE3 received *Eruca sativa* leaf extract, sodium selenite, local nanoselenium, and oral nanoselenium 0.25 mg/kg, respectively; LH and FSH levels did not decrease even in the nanoselenium groups, and these results may be ascribed to a number of variables, including the possibility that the supplied dosages of nanoselenium may have been insufficient to exert noticeably decreasing effects on LH and FSH rank (Odnowe et al., 2021). Moreover, interactions with other hormones and individual variability in treatment responses could have contributed to the minimal hormone level changes. The duration of the study's research may also limit the ability to observe more substantial alterations in LH and FSH values (Eendebeak et al., 2018).

Regarding sperm indices, in the present study Group B showed a significant increase in dead and abnormal sperm (double head, flattened head, reduced hook or banana head, bent neck, bent tail, and multiple abnormalities were examined) with a significant decrease in normal ones compared to Group A due to KET induced-testicle damage by the various mechanisms mentioned above. At the same time, these findings were re-repaired through the administration of bioactive substances, including *Eruca sativa* leaf extract, sodium selenite, and nanoselenium (in dose and route-dependent manner). Groups BE3, BE2, and BE1 exhibited a highly significant increase in normal cells compared to groups B, BC, and BD with a highly significant decrease in the abnormal number of dead sperm compared to B, BC, and BD. PF-SeNPs tended to exert more increment in sperm indices than micro selenium. These results supported the biochemical hormone parameters mentioned above, which showed positive changes in nanoselenium groups; these results can be attributed to the incredibly small dimensions of the nanoparticles, which enable them to easily pass through biological membranes and accumulate in the blood and other tissues (Hoshyar et al., 2016). Except for Group BE1, which showed negligible variations in the quantity of normal cells in comparison to Group BD. To put it another way, the same dose of oral sodium selenite and local selenium nano emulsion has similar effects on sperm morphology, which indicates that they have similar plasma concentrations. Nano emulsions are widely utilized as drug carriers to improve the absorption of
several medications (Morsi et al., 2017). Additionally, the frequency and overall dosage of the systemic drug were reduced during the therapy period to lessen the negative effects of the medication (Gaber et al., 2023).

Although groups BC and BD showed a significant increase in sperm count compared to Group B, all PF-SEnPs subgroups showed a highly significant boost in sperm count in correlation to Gr. BC and BD, with the highest level observed in BE3. In addition, BC (0.5 mg/kg), whereas at higher doses of approximately (1 mg/kg), they could increase spermatoxotoxicity by disrupting cellular, and enzymatic pathways. Additionally, the results showed that sperm motility could be negatively impacted by non-optimal dosages of PF-SEnPs (0.1 mg/kg), which can also confuse the tests and induce oxidative stress and sperm DNA damage (Seyedi et al., 2021).

When matched with groups B and BC, highly significant increases in sperm motility were seen in BD, BE1, BE2, and BE3; in addition, BC significantly increased when compared to Gr. B. These findings will support the beneficial effects of selenium on sperm motility and activity in both its micro- and nanoforms. According to research, sperm motility and viability have been linked to mitochondrial membrane potential (MMP) (Ghafarizadeh et al., 2018). By raising MMP, selenium supplementation protects spermatozoa from mitochondrial harm. In addition to its function as a cofactor to prevent fatty acid oxidation and maintain the integrity of sperm membranes, selenium is also necessary for the GPX enzyme structure. This protects sperm plasma membrane from oxidative damage and the production of MDA on sperm membrane, which improves motility and viability (Dorostkar et al., 2012).

Conclusion

The current research highlights the significance of nanoselenium as a potentially effective treatment agent for enhancing sperm motility, viability, and count, and hence, male fertility. To completely understand the underlying mechanisms and evaluate the long-term effects of nanoselenium treatment on sperm indices and fertility hormones, achieving the goal of respecting male reproductive health, more research is necessary.

Authors declare no conflicts of interest.

References


