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Research Article

Protective Role of Garlic Extract Against Toxicity of Nickel Nitrate on Spermatocytes and Fertility Outcome

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Nickel, Nitrate, Garlic, Mice, Infertility, Healthy lifestyle

Abstract | The current experiment was designed to detect the protective role of garlic extracts against the toxic effects of Nickel Nitrate ($NiNO_3$) in white mice. Thirty male mice, were randomly distributed in five groups: (Group I) as control; other groups were orally administered different doses of $NiNO_3$ daily with diet and water for 30 days through gavage (Group II: 7.1 mg/Kg), (Group III: 13.6 mg\ kg), (Group IV: 13.6 mg\ kg. + Garlic 120 mg\ kg), and (Group V: 13.6 mg\ kg + Garlic 180 mg\ kg). Spermatozoa of male mice were evaluated for count, motility, validity, sperm head abnormality, and live embryos in healthy female mice mated with males treated with $NiNO_3$. Results reported in male mice show that $NiNO_3$ substantially decreases sperm parameters (count, motility, and live spermatozoa) in groups II and III (low and high dose, respectively) compared to the control group. Garlic extract substantially enhanced sperm parameters in group V (with a high dose of garlic) related to group III (without garlic). Sperm count rose considerably in group V compared to group III. The mean sperm head abnormalities in group III were substantially higher than in groups I, II, IV, and V. Groups IV and V (with garlic extract) displayed no significant difference from group I, while mice subjected to $NiNO_3$ showed more significant abnormalities in spermatozoa heads with increasing dosages. Female mice mated with males exposed to $NiNO_3$ showed significantly decreased live embryos in group III compared to groups I, II, IV, and V. This study proved the protective role of garlic extract against the toxicity of $NiNO_3$ on sperm mice, and confirmed the toxic properties of $NiNO_3$ on sperm, leading to infertility in male mice.

Novelty Statement | This study provides novel evidence for the effects of $NiNO_3$ on fertility and living embryos, revealing that $NiNO_3$ causes infertility by affecting the quantity and quality of viable sperm during sperm formation and embryo development, which is treated by garlic extract.

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Introduction

The effects of harmful substances are currently a main focus of interest because they are everywhere (Mahdi *et al.*, 2021). Nickel (Ni) is an essential trace element; modifications in the concentration of Ni have an influence on the construction and activity of hormones like prolactin, adrenaline, noradrenaline, and aldosterone. Ni toxicity influences membrane characterization variations and oxidation/reduction systems in cells. In animals, Ni toxicity causes cancer and leads to embryotoxicity, while the deficiency of Ni is linked to a reproductive rate, decreased development, and changes in lipid and glucose metabolism (Samal and Mishra, 2011). Ni overdose has been found to be a severe poison in the past few years (Rizwan *et al.*, 2022). Nickel and nickel compounds are classified as carcinogens

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(Gathwan *et al.*, 2013; Buxton *et al.*, 2019).

Nickel nitrate ($NiNO_3$) is classified as hazardous, but the data about $NiNO_3$ acute toxicity are limited. This compound of nickel has bioavailability and bioaccessibility, releasing (Ni) ions into biological fluids (Henderson *et al.*, 2012). Nickel nitrate has toxic action on rats' kidney tissue, resulting in kidney cell atrophy (Varsha and Singh, 2021). Nickel oxide (NiO) was found to be a mutagenic agent, toxic, and essential trace element for all multicellular organs (Ali and Al-Mammar, 2024; Abbasa *et al.*, 2024). Wyrobek *et al.* (1983) used sperm head morphology in mice as an index of mutagens or carcinogens. Nickel sulfate administered orally to adult male mice results in a strong reduction in body weight and sperm abnormalities associated with a decline in sperm mortality and sperm count (Pandey *et al.*, 1999). Regularly, nitrite is employed as an addition to food conservation. Still, inordinate nitrite may be harmful and cause either acute or chronic poisoning (Sindelar *et al.*, 2012). Time-dependent mouse infertility induced by nitrite exposure may be caused via apoptosis and oxidative stress (Wu *et al.*, 2022).

Garlic (*Allium sativum*), a multifunctional crop, is appreciated for its therapeutic properties through its bioactive components of allicin, an extensively studied chemical, including phenolic compounds, organic sulfides, polysaccharides, and saponins (Jasim *et al.*, 2023; Jain *et al.*, 2025). Garlic components, with vital oil, matured garlic extract, matured black garlic, and garlic powder, have anti-inflammatory, antioxidant, antiviral, antibiotic, anticancer, anti-hyperlipidemic, and hypertensive properties (Jain *et al.*, 2025). Garlic extraction protects testicles and boosts sperm (Shukry *et al.*, 2021). Garlic, which directly affects estrogen and subsequently affects testosterone, may expected to stimulate sexual cells and sex hormones (Thuy *et al.*, 2020; Hamed *et al.*, 2021).

The study was intended to observe the protective effect of garlic extracts against the toxicity of $NiNO_3$ on white mice. It also demonstrates the toxicity of nickel nitrate ($NiNO_3$), which causes infertility in male mice, by evaluating sperm changes in morphology, count, motility, and validity.

Materials and Methods

Preparation of aqueous garlic extract

Fresh garlic was used, and the aqueous extract was obtained using a modified approach. After homogenizing thirty grams of garlic in one hundred milliliters of cold distilled water, the liquid was repeatedly filtered through cheesecloth and centrifuged at 200 g for ten minutes to produce the clear supernatant. Every mouse received a supernatant daily by oral gavage, based on weight (Martha *et al.*, 1998).

Animals housing

The experiments began in March and ended in June 2024. The animals purchased from the National Centre for Drug Control and Research (NCDCR), Iraqi Ministry of Health, thirty (30) male mice weighing 32–36 g at the age of 8–10 weeks, with six female mice for mating. They were left for seven days to enable acclimation prior to beginning the therapy under regulated temperature settings of 22±3°C and 12:12 light and dark cycle. Mice pellets and tap water were given to animals for eating and drinking.

Sample size

G*Power 3.1.9.7 (Franz Faul software) uses 8% study strength, 0.05 significance, and 0.5 Cohen's D middle effect size. A total of 27 animals were studied. Cohen's D effect sizes are: Small= 0.2, Medium= 0.5, Large= 0.8 (Cohen, 1988).

Experiment design

After one week of the adaptation period, A total of 30 healthy adult male mice were divided randomly into five main groups, with (6) mice in each group; the control group was fed only on a normal diet and water, and the other four groups were orally administered two doses of $NiNO_3$ daily with diet and water for 30 days through gavage and two doses of garlic extract as follow (Pandey *et al.*, 1999):

- Group I served as the control group that received water and standard pellets as food.
- Group II received $NiNO_3$ 7.1 mg/ kg body weight.
- Group III received $NiNO_3$ 13.6 mg/ kg body weight.
- Group IV received $NiNO_3$ 13.6 mg/ kg + garlic120 mg/ kg body weight.
- Group V received $NiNO_3$ 13.6 mg/ kg + garlic180 mg/ kg body weight.

Sperm suspension preparation

After thirty days of exposure to $NiNO_3$ and after twenty-four hours from the last day of treatment, four mice from each group were killed, and the remaining two live mice were left for fertility testing. Collect sperm samples by mixing the sperm from the vas deferens with 1 ml of standard water (0.9% NaCl) in a small dish. To stop sperm adhesiveness, the sperm suspension was kept at 37°C for ten minutes (Otitoloju *et al.*, 2010).

Seminal fluid analysis

Motile spermatozoa were assessed using a Neubauer hemocytometer and a light microscope at 40× magnification with a drop of the sperm suspension solution. Sperm motility was evaluated as a percent of motile sperm of the whole sperm counted (Linder *et al.*, 1986). The technique suggested by Evans and Maxwell helped to ascertain the approximate live-dead ratio of sperm cells (Evans and Maxwell, 1987).

Table 1: Effect of $NiNO_3$ and garlic extract on spermatocyte parameters of male mice in groups of experiments.

Group/parameters	Sperm cell count ($x10^6$/ mL)	Sperm motility %	Live spermatozoa %
Group I (Control group)	152.92 ± 3.1	89.20 ± 2.5	85.21 ± 2.8
Group II ($NiNO_3$ 7.1 mg\ kg)	137.6 ± 3.4	76.8 ± 3.7	76.7 ± 2.8
Group III ($NiNO_3$ 13.6 mg\ kg)	101.3± 4.6	70.3 ± 2.7	72.1 ± 2.9
Group IV $NiNO_3$ 13.6 mg\kg + garlic120 mg\ kg	148.5±1.7	75.1 ± 2.1	79.6 ± 2.5
Group V $NiNO_3$ 13.6 mg\ kg + garlic180 mg\ kg	153.3 ± 2.5	87.1 ± 2.1	86.4 ± 2.8
p-value	0.021	0.032	0.038

The data expressed in mean ± standard deviation (Mean ± S.D); significant p-value < 0.05

The assessment of sperm abnormalities was conducted by preparing smears from the sperm solution. A drop of sperm sample was placed on a clean slide, let dry, and then fixed by ethanol for one hour. The sample was treated with Giemsa for 15–20 minutes, then washed and dried. After that, it was examined under a light microscope at 100× magnification to count the normal and abnormal sperm percentages (Otitoloju *et al*., 2010).

Fertility outcome test

A fertility test was done by using the surviving mice from each group. After 24 hours after the final day of treatment, the treated males were paired with untreated healthy females overnight in a ratio of one male to three females. Every female was housed alone when the vaginal plug was noted. Pregnant mice were immolated at eighteen days of gestation, and embryos were separated from the uterus to determine the number of live and dead implants (Sasi *et al*., 2023).

Statistical analysis

The statistical analysis system (SAS Software, 2021) used a one-way analysis of variance (ANOVA) to evaluate the significance level between the control and treatment groups, with $p < 0.05$ indicating statistical significance. The data were expressed as mean ± standard deviation (mean ± SD).

Results

The observation reported that in $NiNO_3$-treated mice, there was a reduction in food and water consumption, relative to the control group; moreover, the decrease was dose-dependent. The current work used ANOVA to demonstrate that $NiNO_3$ at varying dosages (in groups II and III) substantially reduced sperm parameters, including sperm count, sperm motility (%), and live spermatozoa (%), in male mice compared to the control group (group I) ($p < 0.05$). The most pronounced toxicity of $NiNO_3$ was seen in group III ($NiNO_3$: 13.6 mg/Kg), whereas group II exhibited lower toxicity ($NiNO_3$: 7.1 mg/Kg) in comparison to the control group, as shown in Table 1. However, the effect of garlic extract appeared in group V, with the sperm parameters [sperm count, sperm motility (%), live spermatozoa (%)] increased significantly ($p < 0.05$) related to the parameters in group III (with $NiNO_3$ 13.6 mg\ kg), but no vital difference in the groups IV (with $NiNO_3$ 13.6 mg\ kg + garlic120 mg\ kg) and V (with $NiNO_3$ 13.6 mg\ kg + garlic180 mg\ kg) related with a control group. About the differences between groups III (without garlic extract) and IV (with garlic extract), all parameters exhibited non-significant differences ($p > 0.05$) except sperm count, which increased significantly ($p < 0.05$) compared to the count in group III.

In Table 2 the ANOVA test showed the sperm head abnormalities in group II mean value (9.9 ± 1.6) were non-significant ($p > 0.05$) compared to group I mean value (6.80 ± 1.3), but in group III, the mean value (18.7 ± 1.9) elevated significantly ($p < 0.05$) related to group I, II, IV, and V (6.80 ± 1.3, 9.9 ± 1.6, 8.3 ± 1.7, and 7.6 ± 1.70) respectively. In group IV with garlic extract, 120 mg\ kg, the mean value (8.3 ± 1.7) and group V (7.6 ± 1.70) were non-significant ($p < 0.05$) with group I (6.80 ± 1.3), and the abnormalities of the head of spermatozoa of male mice exposed to $NiNO_3$ were increased in abnormalities with multiplication of $NiNO_3$ doses. The abnormalities were in some arbitrary types (amorphous, hammer-shaped, cylindrical, spinal, triangular, oval, curved, and double-head sperm) (Figure 1).

Table 2: The effect of $NiNO_3$ and garlic extract on spermatocytes, with the abnormalities in the head in each group.

Group	The mean of an abnormal head of sperm
Group I	6.80 ± 1.3
Group II	9.9 ± 1.6
Group III	18.7 ± 1.9
Group IV	8.3 ± 1.7
Group V	7.6 ± 1.70
p-value	0.018

The data are expressed in mean ± standard deviation (Mean ± S.D); significant p-value < 0.05.

The mean live embryos in female mice mated with males exposed to $NiNO_3$ were revealed in Table 3, and there was a substantial ($p < 0.05$) decline in the number of

live embryos in group III mean value (2.83 ± 2.1) treated with $NiNO_3$ in high dose (13.6 mg\ kg) comparing with group I, II, IV, and V (10.30 ± 1.4, 7.37 ± 1.6, 8.42 ± 1.3 and 9.82 ± 2.6) respectively, and non-significant value ($p > 0.05$) in the number of live embryos in group V (13.6 mg\ kg + garlic180 mg\ kg) compared to group I (10.30 ± 1.4) mean value. Table 3 and Figure 2 demonstrate the effect of $NiNO_3$ and garlic extract on live embryos.

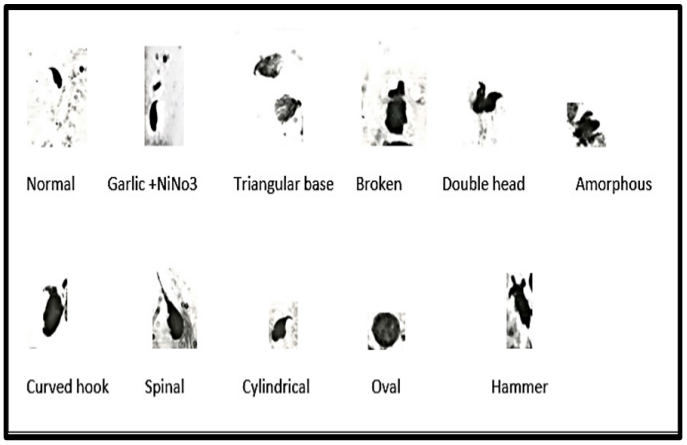

Figure 1: Glass smear of seminal fluid shows ahead of each spermatocyte for description of head deformities, Giemsa stain 1000x.

Table 3: The mean number of live embryos in female mice mated with male mice treated with $NiNO_3$ and garlic extract.

Group	The mean number of live implants
Group I	10.30 ± 1.4
Group II	7.37 ± 1.6
Group III	2.83 ± 2.1
Group IV	8.42 ± 1.3
Group V	9.82 ± 2.6
p-value	0.032

The data expressed in mean ± standard deviation (Mean ± S.D); significant p-value < 0.05

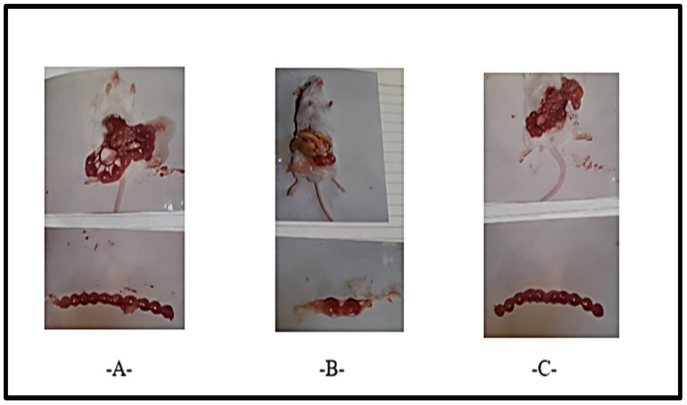

Figure 2: Live and dead embryonic implants after mating of a normal female with $NiNO_3$ treated male. A, Control; B, $NiNO_3$; C, $NiNO_3$+ Garlic. The figure shows the number of embryos in the abdomen of female mice and embryos separated from female mice.

Discussion

The current study's results improved the influence of Nickel (Ni) on spermatogenesis and fertility. This finding is in accordance with previous studies demonstrating that Nickel and Nickel's compounds had a negative effect on spermatogenesis in male mice. Still, to our knowledge, no studies have considered the impact of nickel nitrate on mice fertility.

The experimental study conducted by Zeng *et al.* (2023) reported that nickel chloride ($NiCl_2$) caused abnormalities in the testis and epididymis histopathological structure and depletions in sperm count with an increase in germ cell shedding in the lumen, indicating that $NiCl_2$ is able to cause damage to the critical testicular structure and the blood-testis barrier (BTB) which is sensitive to reproductive toxicity of a variety of environmental contaminants. Another study by Pandey *et al.* (1999) on a different nickel compound showed that oral treatment with nickel sulfate ($NiSO_4$) induces significant histological alterations in the testes and epididymis and reduces sperm in mice.

Prior studies demonstrated that nickel can build up in the testes, causing cell death, abnormal sperm formation, and spermatogenesis, which can harm reproduction in mice. It also affects (BTB), which provides a constant environment for spermatogenesis (Zeng *et al.*, 2023). The results by Forgacs *et al.* (2012) found that nickel is toxic to the testes. However, the findings of Yang *et al.* (2021) demonstrated that various dosages of $NiCl_2$ administered to mice may gather in the testes and disrupt the hypothalamic-pituitary-testicular axis, which plays a pivotal role in sperm production abnormalities in mice caused by $NiCl_2$.

However, Zemanova *et al.* (2007) found that Ni affects the cyclic nucleotide-gated channels, which are vital to sperm functions. They noticed that a lack of Ni caused a big decrease in the motility and amount of sperm in the epididymis, epididymal transportation time of sperms, and testes' sperm origination rate. Lack of Ni also reduced the weight of the seminal vesicles and prostate glands to a substantial extent.

About the effect of nitrate on fertility, research by Wu *et al.* (2022) reported that nitrate exposure may lead to infertility in mice, revealing further effects with repeated exposure. Previous studies by Amini *et al.* (2017, 2018), indicated the impact of nitrate on male mice, utilizing both direct and indirect assessments of sperm parameters, which suggested damaging effects displayed as decreased sperm motility, lowered sperm count, elevation of total abnormal sperm, and diminished testicular enzyme activity. Research on rabbits exposed a negative correlation between nitrate

exposure and embryo count, testosterone levels, and sperm restrictions (Pant and Srivastava, 2002).

There is agreement with other studies regarding the embryos in female mice after mating with male mice treated with $NiNO_3$. Greenlee *et al.* (2004) reported an elevated percentage of spontaneous abortion, embryonic demise, or heightened death in the embryos of female mice subjected to nitrate. Furthermore, additional research has shown links between nitrates and inborne with low weight, tiny for gestational age, premature delivery, stillbirth, and adverse reproductive outcomes (Ward *et al.*, 2018). According to different research, around 30% of pregnancies terminate spontaneously; this might be because of poor semen quality, the causes of which are not well known (Weselak *et al.*, 2008).

Concerning the findings of this study, verifying the constructive impact of garlic extract on sperm quality, motility, and viability, and against the toxicity of $NiNO_3$, most studies agree with these results. A survey by Rabbani (2019) showed that garlic powder at a dosage of 150 mg/Kg mitigated the cytotoxic effects of $NiCl_2$ on somatic and germinal cells, suggesting that it is associated with free radical scavenging activities. Garlic enhances male sexual function and positively influences the recovery of testicular functions, highlighting its beneficial effects on sperm concentration, motility, and viability (Bahrami *et al.*, 2014; Qadariah *et al.*, 2020).

Conversely, the current study's findings contradict those of Qian *et al.* (1986), who stated that rats treated with garlic displayed a decline in sperm quality and functionality. The primary distinction and reasons for varying outcomes may be from the absence of constancy among study models and the disparate quantities of garlic administered to experimental subjects; nonetheless, providing more garlic supplementation over an extended duration to adult rats resulted in an increase in epididymis spermatozoa (Green *et al.*, 1985).

Garlic includes phytoestrogens that directly influence estrogen, a precursor to testosterone development, suggesting that garlic may impact sexual cells and sex hormones (Hammami *et al.*, 2013). Garlic complements enhance luteinizing hormone (LH) production from the pituitary gland, hence stimulating testosterone output from the testes (Oi *et al.*, 2001). Cooked garlic is indicated to have superior corrective properties, while impacting the proliferation of sperms in the epididymis and testicles and enhancing spermatogenesis (Bahrami *et al.*, 2014).

Garlic comprises components such as flavonoids, vitamins, fructose, and sulfur compounds, which may assist in removing free radicals. Sulfur compounds in garlic protect spermatogenesis, possess antioxidant characteristics, and may enhance fertility by diminishing lipid peroxidation (Moher *et al.*, 2009). Garlic is recommended to help as a therapeutic for infertility. A study by Asadpour and others found that garlic has antioxidant properties because of vitamin E, which helps block oxygen peroxide (Asadpour *et al.*, 2013). Nasr's findings indicated that garlic's antioxidant possessions may decrease the toxicity of destructive medications on the testes and enhance fertility and sperm production (Nasr, 2017).

To our knowledge, there is no research regarding the effects of $NiNO_3$ on mice fertility and living embryos from mating between male mice treated with $NiNO_3$ and healthy female mice. The findings of this study recorded that $NiNO_3$ caused infertility, as fertility based on the quantity of viable sperm with good quality during sperm formation to produce healthy embryos, and consequently garlic extract (including its components) can be considered a respectable treatment for infertility cases resulting from pollutants such as nickel, nitrate, and other compounds, as it is an antioxidant and prevents toxicity by mechanism may be associated with the free radical removal activity.

Conclusions and Recommendations

This study proved the garlic extract's protective effect, at high doses, against the toxicity of Nickel Nitrate on sperm mice. Furthermore, it confirmed the toxic effects of Nickel Nitrate on sperm count, morphology, fertility, and embryos, leading to the infertility of male mice.

Future research must include molecular or biochemical assays to understand mechanisms, such as antioxidant levels or DNA damage markers.

Declarations

Acknowledgment

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IRB approval

All animal procedures were conducted in accordance with the rules of the Animal Care and Use. Ethical approval for this study was arranged by the Institutional Review Board (IRB) of the College of Dentistry, University of Baghdad, under project No. 1008825.

Ethical statement

All experiments and procedures were approved by the Ethical Review Committee of Baghdad University, College of Dentistry, which permitted this experimental study under No. (1008).

Limitations of the study

More animals are needed to increase the confirmation

of the results, and they need long-term evaluation.

Generative AI and AI-assisted technology statement

No Generative AI and AI-assisted technologies were used in the writing process .

Conflict of interest

The authors have declared no conflict of interest.

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