Kerbala Journal of Veterinary Medical Sciences Issue (2), Volume (2), (2026)

The Therapeutic Effect of Alcoholic Extract, Nano-particles and Endophytic Fungi of *Eruca sativa* on Male Rats reproductive Hormones Dysfunction

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Received: 12/4/2026 **Accepted:**21/4/2026 **Published:** 15/6/2026

***Abstract*—** The present research examined whether *Eruca Sativa*'s alcohol extract, *Eruca Sativa*'s nanoparticles, and Endophytic fungal extracts could prevent reproductive hormone dysfunction caused by lead acetate in male rats. Data collection occurred from Oct 2025 – Feb 2026 at the College of Vet Med, University of Kerbala, Iraq. Adult male rats (8-10 wks old) weighing between 200-250 grams were randomly assigned to one of six experimental groups: Negative Control, Positive Control (lead-exposed), Alcohol Extract Group (*E. sativa*), Nanoparticle Group (*E. sativa*), *Aspergillus* Endophyte Group, and *Fusarium* Endophyte Group. Serum Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), Testosterone, and Inhibin-B were determined through ELISA assays. The highest amounts of phenolics were detected in the alcohol-based extraction solutions. Due to their small size, nanoparticles retained an appreciable number of the bioactive compounds. Treatment with Lead Acetate produced significant reductions in serum concentrations of LH, FSH, and testosterone when compared to the negative control. Significant increases in serum Inhibin-B were observed as well. Treatments with each of the four test agents resulted in statistically significant improvements in serum FSH levels. Statistically significant improvements in LH levels were achieved with both *Fusarium* Endophyte and *E. Sativa* Nanoparticles. The greatest improvement in testosterone levels was noted in animals treated with *E. sativa* nanoparticles. Additionally, testosterone was increased in animals treated with Fusarium endophytes. No increase in testosterone levels was seen in either of the two other treatment groups. Statistically significant decreases in serum Inhibin-B were observed in all four treatment groups.

***Keywords* —** *Eruca sativa, Endophytic Fungi, LH, FSH, Male reproductive system*

INTRODUCTION

The male rat reproductive system includes the testes, epididymis, vas deferens, seminal vesicles, prostate gland and penis. Each component works in tandem to create, store and transport sperm for reproduction purposes (1). In addition, the male reproductive system contributes significantly to an individual's general health and well-being. However, over recent years, it appears that male reproductive problems are becoming increasing common which could contribute to increasing public concern regarding the many different chemicals and materials that may potentially influence male reproductive health (2).

Over the past few decades, the process of discovering new drugs to cure specific illnesses was very time-consuming and expensive and posed considerable risks to both the pharmaceutical companies and patients. Consequently, because of the potential of creating cheaper, less hazardous methods of finding new medicines, pharmaceutical companies are now starting to explore using herbal remedies and other plant-based items as alternative sources for medicine. Synthetic pharmaceuticals have several disadvantages including unwanted side effects, and therefore, the development of medications that can be used instead of conventional synthetic pharmaceuticals with fewer or no negative side effects is needed (3). As such, there has been substantial scientific investigation of medicinal plants in terms of their efficacy in treating various diseases along with the processes involved in extracting and analysing the compounds found in medicinal plants for their ability to treat various types of illness as well as understand how they function as medication (4).

Eruca sativa (*E. sativa*) also referred to as rocket, is an edible leafed crop that grows worldwide (5). Rocket contains vitamins A, B6 and C, beta-carotene, lutein, zeaxanthin, cryptoxanthine and flavonoid content (6). Therefore, the use of rocket as a model in either animal or human studies examining testicular function/histology is considered. Additionally, considering whether rocket may be used as a

therapeutic agent to either treat or prevent disease conditions, specifically those related to male reproductive health (7).

Flavonoids are among the largest classes of naturally occurring phenolic compounds that are produced during secondary metabolic processes in plants (8). This widespread interest in medicinal plants has prompted researchers to investigate how medicinal plants affect male fertility through the existing body of literature. Some medicinal plants enhance sperm count/motility whereas numerous medicinal plants induce changes in hormone secretion by the testicles (9).

Endophytic organisms inhabit internal tissues of medicinal plants. These endosymbionts generally include fungi and bacteria that reside inside internal plant tissues without harming the host. They can also increase biosynthesis of bioactive compounds by the host (10). For example, endophytes grow intra- or intercellularly within parts of plant tissues such as roots, shoots, leaves and/or seeds for at least part of their lifecycle where they form symbiotic relationships (11). Nano-Medicine is an area of study of medicine based on nano technology. It has been possible to synthesize the nanoparticles (NPs) and utilize them in different medical fields so as to prevent, diagnose, treat and also control diseases (12). The present study, aimed to the asses the efficacy of synthesis nanoparticles of Eruca sativa, extract of Eruca sativa and the endophytes in the plant in the management of dysfunction of male reproductive hormones.

MATERIALS AND MTHODS

The research was conducted at the College of Veterinary Medicine/University of Kerbala, from October 2025 to February 2026, involving 40 adult male rats aged between 8 to 10 weeks, with an average weight ranging from 200 to 250 grams. Following a 15-day acclimatization period in metal cages under controlled environmental conditions (temperature about 23±2 °C, light/dark cycle conducted by12 hours for each); with unrestricted access to food and water, the rats were randomly divided into six groups, each containing five animals, as outlined below:

-Experiments Design: -

The animals in current study were divided into six groups. Each group consists of five male rats used as the following:

1-GroupI: (negative control group): five male rats were received D.W daily for 5 weeks

2- Group II: (positive control group or lead acetate group): For seven days, animals in this group received intraperitoneal injections of lead acetate at a dose of 30 mg/kg B.W. every day. Depended on LD50 (13).

3- Group III: After testicular dysfunction by lead (7 days) therapeutic dose of *E. Sativa* extract (200 mg/kg/day) orally via gavage tube once daily for 30 days (14).

4- Group IV: After testicular dysfunction by lead (7 days) therapeutic dose of *E. Sativa* nanoparticles (0.5 mg/kg/day) via gavage tube once daily for 30 days (15).

5-Group V & VI: After testicular dysfunction by lead (7 days) therapeutic dose of endophytes extract (30 mg/Kg/day) orally via gavage tube once daily for 30 days of the extract of *Aspergillus* and *Fusarium* (16).

-Parameters Studied:

After the end of the experiment blood serum was taken for the laboratory tests, to detect the levels of sexual hormones: Luteinizing hormone (LH), Follicle-stimulating hormone (FSH), Testosterone and inhibin). Using ELISA and the specific kits provided by (Sunlog/China).

-Alcoholic extract of plants:

The plant material was first washed, dried (in shaded areas) and milled to a fine powder. Powdered material was extracted from the plants with an alcohol-based solvent — most commonly 70% ethanol — through a process of cold extraction called maceration over 24-72 hours or even longer periods at room temperatures while the solution is shaken periodically. After the materials have been mixed together, they were strained and the solvent was removed using a vacuum evaporator as the solution was cooled to remove the crude alcoholic extract that would be placed in airtight storage at 4°C until it was used (17).

-Nano-particles preparation:

Chitosan serves as a stabilizing agent due to its cationic nature and ability to cap nanoparticles. Extract Eruca sativa contains polyphenols and flavonoids that can act as reducing agents, and contribute to the surface plasmon resonance (SPR) characteristics of the final nanoparticles (18). The following techniques were used to confirm nanoparticles formation, like Scanning Electron Microscope (SEM), X-ray Diffraction (XRD) Analysis, Atomic Force Microscopy (AFM) Analysis, High Performance Liquid Chromatography (HPLC), Fourier Transforms Infrared Spectroscopy Analysis (FTIR),

-Isolation of endophytic fungi:

Fungi have been found in all parts of the world. They are often mistaken for plants or animals. Fungi can grow both above ground and underground. Many fungi help the environment by breaking down dead material. Some fungi also break down pollutants. Fungi play an important role in decomposing organic matter. Without decomposition, organic materials would continue to accumulate and create large amounts of waste. Decomposition is one of the most important processes that occur in nature. It occurs in every ecosystem around the world and it supports life decomposition begins when organisms die and their bodies start to decay. The process involves microorganisms like bacteria and fungi consuming and breaking down dead organic matter. This process helps return nutrients back to the soil, allowing other living things to consume them. (19).

-Determination of total phenolic compounds:

The total amount of phenolic compounds was determined in the ethanolic extract with a standard Folin -Ciocalteu reagent. 3 gm of sample extracted with 100 ml of 70 % ethanol. The reaction mixture contained 2 ml of the extract, and 700 µl of the Folin-Ciocalteu reagent (Merck, Germany) and 1.5 ml of 20% sodium carbonate. The sample was then mixed on a vortex mixer and diluted with distilled water to the final volume of 10 ml. After 2 h reaction, the absorbance at 765 nm was determined and used to estimate the phenolic content using the calibration curve made with gallic acid (Sigma-Aldrich, Germany). The total amount of phenolic compounds was expressed in mg gallic acid equivalent (GAE) per g dry weight.

- **Statistical Analysis**:
The statistical program Graph Pad Prism 8.0 the t-test was used, P≤0.05 was chosen as the standard of significance. The data points were shown as mean± SD.

RESULT AND DISCUSSION

Five major phenolic compounds were identified using reversed-phase HPLC at 280 nm in each of the tested samples, including gallic acid, quercetin, catechol, benzoic acid and salicylic acid, as shown in (Table 1). Each sample had the largest quantity of compounds extracted by alcohol; therefore, this method produced the best phenolic preparation, but the chitosan nanoparticles prepared for use as a carrier system retained a significant amount of the compounds, demonstrating that the bioactive phytochemicals have been successfully incorporated into the nanoscale delivery system (20). The fact that there is less of each compound present in the chitosan particle preparation than in the crude alcoholic extract does not mean that the chitosan particles will be less effective in terms of biological activity. This could potentially be due to the protective effects of the chitosan on the phenolic compounds from degradation and to the increased delivery efficacy afforded by encapsulation of the compounds within the nanocarriers (21).

Table 1. Phenolic compounds identified using reversed-phase HPLC

No	Name	Gallic acid	Quer-cetin	Cate-chol	Benzoic acid	Salicylic acid
1	*E. sativa*-nanoparticles	69.35	47.8	41.02	22.51	39.8
2	*E. sativa* extract	100.25	90.36	72.56	48.98	84.25
3	*Aspergillus*	44.65	21.6	19.8	11.4	20.3
4	*Fusarium*	50.33	23.5	21.7	13.8	23.6

Microscopic examination of the fungal isolates was conducted. Two different morphotypes were observed in the isolates. Morphotype two displayed a dark, globose conidial head at the apex of a conidiophore containing many spherical conidia; characteristics indicative of *Aspergillus* spp. In comparison, morphotype one contained hyaline, septate hyphae and had elongated, slightly curved to falcate, multicellular conidia; characteristics that are indicative of *Fusarium* spp. Based on these characteristics, it is likely that the two isolates represent *Aspergillus* and *Fusarium* endophytes, respectively, as shown in (Figure 1).

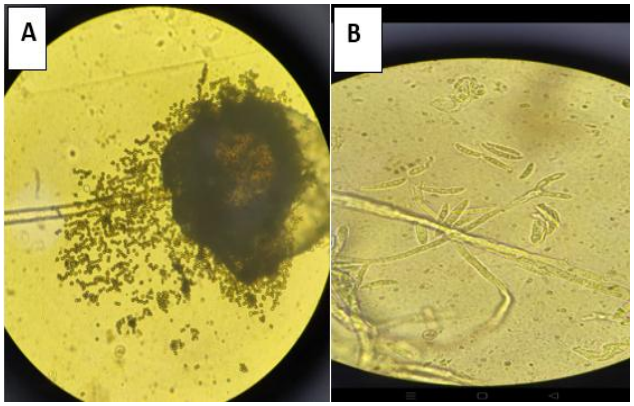


Figure 1. Microscopic characterization of tow types of endophytic fungi A) *Aspergillus spp*., B) *Fusarium spp.*

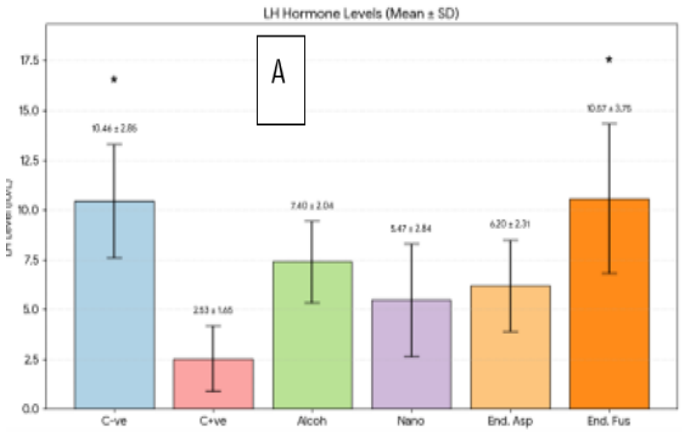
The effect of *E. sativa*, *E. sativa*-nanoparticles, and endophytic fungi (*Aspergillus* and *Fusarium*) on the reproductive hormones LH, FSH, testosterone and inhibin B comparing with the control groups showed in (Figure 2: A, B, C and D) respectively, and the results revealed that, there were significant differences among groups under study. As seen in (Figure 2: A), LH, treatment effects are most clearly evident as a comparison of each treatment to the lead acetate induced positive control (c+ve). Only the *Fusarium* endophyte group demonstrated an elevated level of LH which was statistically significant when compared to the lead acetate positive control (+ve) group; thereby demonstrating a level of LH restoration that is comparable to the lead acetate negative control (-ve) group.

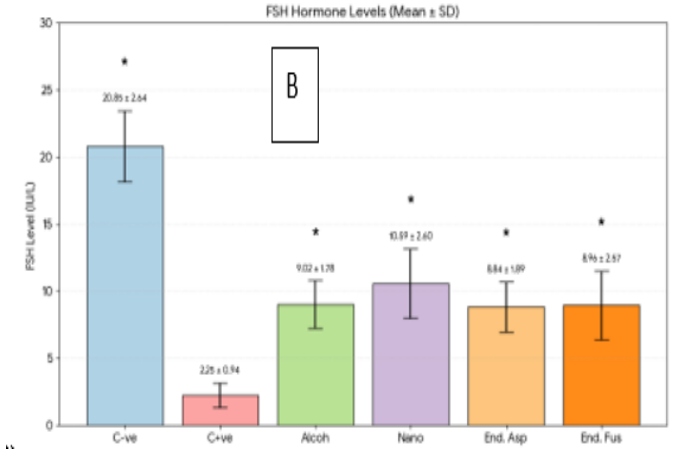
Overall, there was a highly significant difference among the treatments based on ANOVA and Post-Hoc Tukey Honestly Significant Difference (HSD) analysis it was determined that only two of the treatments, *Fusarium* group ($p = 0.0019$, 10.572 ± 3.748) and (c-ve) group ($p = 0.0022$, 10.456 ± 2.850) were significantly different than the lead acetate positive control (c+ve) group.

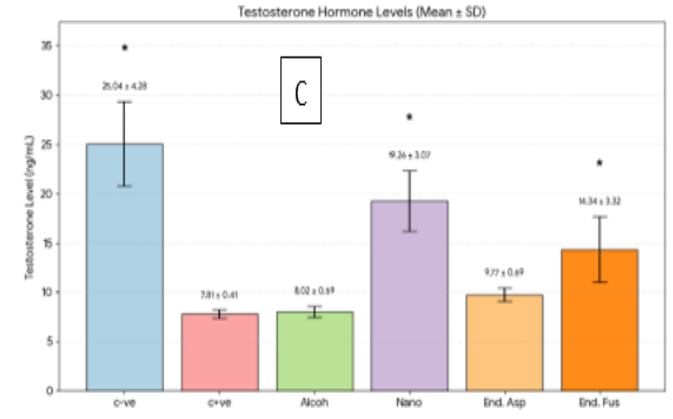
All other treatments *E. sativa* extract (7.398 ± 2.040), *E. sativa*-nanoparticles (5.470 ± 2.837), and endophytic fungi *Aspergillus* (6.196 ± 2.307), exhibited numeric increases, however none of these treatments reached statistical significance relative to the positive control ($p > 0.05$). Validation of lead toxicity effect (c+ve) group; as expected, lead acetate disrupted the hypothalamic-pituitary-gonadal axis causing a marked reduction in LH (2.531 ± 1.647) compared to the lead acetate negative control (c-ve) group (10.456 ± 2.850).

This confirms the disruptive effect of lead acetate on the hypothalamic-pituitary-gonadal axis in the rat model (22). On the other hand, specific bioactivity of secondary metabolites that produced by *Fusarium* may be involved in a novel or more potent mechanism of action for stimulating LH release (23). A major reason why the *Fusarium* group is the most likely to be responsible is due to the fact that LH is a hormone that varies biologically; the *Fusarium* group most likely elicited the largest and most consistent effect size. While, the other groups demonstrated only partially elevated levels that were either too small, too variable among the animals, or both,

to cross the statistical threshold to demonstrate a significant elevation in LH (24).







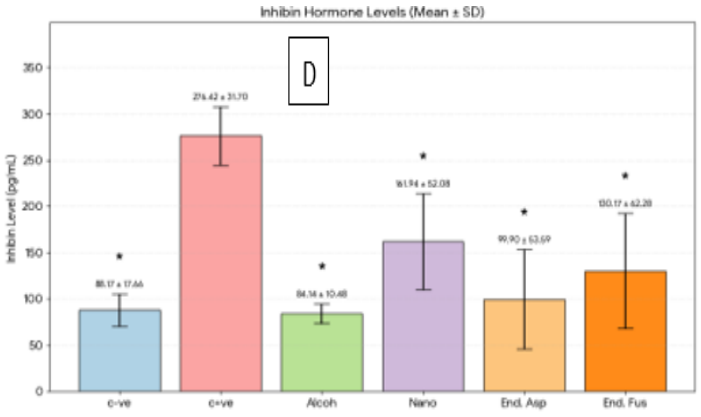


Figure 2. The effect of *E. sativa*, *E. sativa*-nanoparticles and Endophytic fungi (*Aspergillus* and *Fusarium*) on the LH (A), FSH (B) testosterone (C) and inhibin (D). P value ≤ 0.05 is significant

The LH is also a variable hormone and it is not a stable such as many chemical markers. It is secreted in a pulsatile manner, therefore the amount of LH present in the blood can vary significantly depending upon the time of sampling and the individual's physiological characteristics (25). Due to the inherent biological fluctuations in LH, a single serum measurement of LH may miss the true average endocrine condition of the animal (26). Consequently, the use of a single serum measurement of LH reduces the statistical power of detecting differences in LH. Thus, some groups of animals may exhibit a statistically insignificant upward trend in LH merely because the LH hormone itself has natural fluctuational properties (23).

For FSH as revealed in (Figure 2: B), lead acetate induction (control +ve) resulted in a marked decrease in FSH levels as compared to the control (-ve). All treatment groups (alcoholic extract, nanoparticles, *Aspergillus* and *Fusarium* endophyte) were found to have significantly elevated FSH levels compared to the induced control (+ve). The statistical analysis showed each treatment group was significantly different from c+ve (2.25 ± 0.94) (*E. sativa* extract $p = 0.0006$, 9.022 ± 1.780; *E. sativa*-nanoparticles $p < 0.0001$, 10.588 ± 2.597; *Aspergillus* $p = 0.0008$, 8.840 ± 1.894 and *Fusarium* $p = 0.0007$, 8.963 ± 2.575), and c−ve (20.85 ± 2.64) was also found to be significantly different from (c+ve) ($p < 0.0001$).

Lead acetate has been shown to be a gonadotoxic agent which affects the (HPG) axis. The large decline in FSH levels confirmed that lead exposure was successful in inducing testicular dysfunction due to oxidative stress mechanisms damaging either the pituitary gonadotropes or disrupting the feedback loops that regulate hormone secretion (27). *Eruca sativa* contains high amounts of antioxidants (flavonoids and phenolic compounds) which reduce oxidative stress caused by heavy metals. Thus, the antioxidant activity of *E. sativa* likely protected the anterior pituitary function thereby partially restoring FSH secretion (14).

The nanoparticles group recorded the largest numerical mean value among the treatments (10.59 ± 2.60) compared to the extract (9.02 ± 1.78). Although the difference between the two was not found to be statistically significant in this data,

there appears to be a pattern that shows the nano-formulation may provide better bioavailability or cellular uptake of the bioactive compounds, a phenomenon commonly seen in studies of plant-based nanoparticles used as drugs (15).

The endophytic fungi *Aspergillus* and *Fusarium* were found to exhibit a notable therapeutic effect. Both significantly increased FSH levels to 8.84 ± 1.89 and 8.96 ± 2.58, respectively. This demonstrates that both *Aspergillus* and *Fusarium* have considerable bioactivity similar to that of their host plants (28). Endophytic fungi are known to produce secondary metabolites (alkaloids and flavonoids) which act like the biological effects of their host plants. The results demonstrate that metabolites produced by *Aspergillus* and *Fusarium* were capable of reducing the endocrine disruption caused by lead. Therefore, they represent an additional possible resource for developing reproductive protection agents (29).

Lead acetate induced significantly lower testosterone levels (c + ve) as opposed to the control (c -ve) groups, Figure (2: C). Of the three treatments tested, nano-encapsulated *E. sativa* and *Fusarium* endophyte were found to be the only two to have a significant restoration of testosterone levels relative to the control (c +ve) group. However, neither the alcohol extract nor the *Aspergillus* treatment had a statistically significant increase in testosterone production.

According to statistical analysis, the overall effects of the five treatments were all highly significant, by the comparisons of each treatment with the positive control. It was determined that testosterone production in the lead acetate treated animals c +ve, 7.811 ± 0.411) was significantly lower than in the untreated controls (c -ve, 25.040 ± 4.282) at (p- < 0.0001). For *E. sativa*-nanoparticles, testosterone was significantly higher (p < 0.0001, 19.257 ± 3.066) than in the lead-treated group. While for *Fusarium*, testosterone was significantly higher (p = 0.0083, 14.336 ± 3.321) than in the lead-treated group. Besides, alcoholic extract there was no statistical significance (p = 1.0, 8.021 ± 0.585). As well as, *Aspergillus* also showed there was no statistical significance (p = 0.88, 9.774 ± 0.689).

Due to lead induced hypogonadism; the large decrease in testosterone in the lead treated animals (c + ve) versus the untreated controls (c -ve) indicates a large reduction in testicular function. It has been previously established that lead accumulates in the testes and inhibits the activity of several steroidogenic enzymes which impairs the synthesis of testosterone in Leydig cells (15).

The advantages of using nanoparticles found that, the *E. sativa*-nano-treatment exhibited the greatest recovery and this was statistically significant and greater than twice the recovery of the alcoholic extracts. These findings indicated that encapsulation into nanoparticles increases the bioavailability and ability of the antioxidant compounds present in the plant to penetrate Leydig cell membranes and protect the steroidogenic enzymes from oxidative damage (30). As well as, different effects of fungal treatment, as in a manner similar to the LH values, the endophytic *Fusarium* exhibited significant bioactivity (mean 14.34), while *Aspergillus* did not. Therefore, these data support the hypothesis that *Fusarium* produces unique secondary metabolites that are more potent in either stimulating the HPG axis or protecting testicular tissue than the products of Aspergillus produced within this same host-fungus interaction (31).

In relation to inhibin B, as in (Figure 2: D), the results demonstrated that lead acetate induced a significant elevation of inhibin B, and that all of the other treatments nanoparticles, alcoholic extract, and both endophyte species significantly decreased the levels of inhibin B, which are at a level similar to that of the untreated healthy control group. Therefore, all treatments had a significant effect on reversing the lead acetate-induced increase in inhibin B at p-value and mean for *E. sativa* alcoholic extract (p< 0.0001, 84.142 ± 10.483), *E. sativa* nanoparticles (p=0.0033, 161.941 ± 52.084) *Aspergillus* (p< 0.0001, 99.896 ± 53.587) and finally *Fusarium* (p= 0.0002, 130.173 ± 62.280).

The levels of inhibin B in the positive control group (c+ve) were greatly increased (276.419 ± 31.702) relative to the levels in the negative control (c-ve) (88.166 ± 17.656). Inhibin B is produced by Sertoli cells and acts to provide negative feedback to the pituitary gland regarding regulation of FSH (32). It has been previously reported that inhibin B will be decreased after a significant amount of testicular damage, but in cases of chronic exposure to toxins or compensation of loss of Sertoli cells, its levels may be elevated and provide evidence of an abnormality in the feedback loop or Sertoli cell hyperplasia or tumorogenesis (27). Typically, however, inhibin B levels would be expected to be decreased in cases of lead toxicity (33).

Treatment effectiveness for all treatments; found there was no specificity found in terms of the type of intervention (extract, nano-particle, fungi). Each of these interventions resulted in a lowering of inhibin B to a level that was statistically equivalent to that seen in the healthy control. Therefore, it appears that the stabilization of Sertoli cell function is a common pathway to restore fertility using *Eruca sativa* and its endophytes (34).

While the effect of alcoholic extract on inhibin B levels, found that, the alcoholic extract appeared to have the lowest mean value (84.14) and therefore appeared to be the most potent of all treatments examined. The contrast of this finding is apparent when comparing it to the testosterone results, where the nano-particle formulation appeared to be the most potent. These findings suggest that the phytochemicals present in the crude extract responsible for normalization of Sertoli cell secretion (i.e., Inhibin B) may be different from those required for recovery of Leydig cell production (i.e., Testosterone) or that they may be more readily available for use in the crude extract (35).

CONCLUSION

The current study demonstrates that lead acetate causes severe reproductive hormonal disruptions in male rats by reducing LH, FSH, and testosterone and increasing inhibin B. Moreover, our results show that the treatments with *Eruca sativa* preparations and endophytic fungal extracts can significantly reduce these disruptions; however, the extent of recovery differs among interventions. Notably, *E. sativa*

nanoparticles produce the largest impact on restoring testosterone levels. Also noteworthy, the *Fusarium* extract has been shown to be very effective for enhancing both LH and testosterone levels. Finally, all evaluated treatments normalize FSH and inhibit B levels. Overall, these results provide evidence that *E. sativa* may serve as a valuable source of novel therapeutic strategies to protect against heavy-metal induced reproductive dysfunction in males.

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