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Histological Estimation for Role *Nigella sativa* in Supporting of Fertility in Mice

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ABSTRACT

The current study investigates the role of *N. sativa* in enhancing the male fertility of mice through evaluation of serum testosterone, sperm motility, sperm numbers and histology of epididymis and testes. Totally, 30 male mice were purchased from the local markets, acclimated for 1 week, and divided equally to control G1 and experimental G2 groups. The findings revealed a significant elevation in values of serum testosterone in G2 (41.39 ± 1.51) rather than G1 (32.16 ± 1.38). Also, the percentage of sperm motility were increased significantly in G2 (78.21 ± 3.48%) rather than of G1 (71.73 ± 4.17). Concerning the number of sperms, slight significant elevation was observed in values of G2 (17.03 ± 1.27) when compared to those of G1 (16.44 ± 1.08). Histologically, the findings of epididymal testicular tissues of G1 were showed a normal architecture; however, histological sections of G2 were enhanced significantly in particular testicular parenchyma with increasing of germinal cell population and spermatocytes. Similarity, the mature and immature Leydig cells numbers were also increased significantly with decreasing in degenerating cells number. However, significant alteration in numbers of fibroblast and spermatogonia was not observed. In conclusion, *N. sativa* is can be used as aphrodisiac and fertility promoting agent in both healthy and diseased individuals. Male infertility or sub-fertility occurs together with abnormal semen; therefore, enhancement of semen quality and quantity can be done with lowered advantages using the *N. sativa*. Moreover studies concerned the long-term utilization of various extracts of the plant and activity on male / female reproductive systems appear necessary to monitor the toxic potential of various xenobiotics.

1. Introduction

Fertility refers to the actual production of offspring rather than the physical capability to produce which is termed fecundity that explained by gamete production, fertilization, and carrying a pregnancy (Zegers-Hochschild et al., 2017; Sterling and Garcia, 2020). In industrialized nations, infertility becomes a top priority for many health organizations due to the widespread condition and decreasing the numbers of offspring (Inhorn and Patrizio, 2015). Male sub-fertility and infertility means a diminished or absent ability to produce descendants due to many causes ranging from hormonal imbalance to physical problems; however, approximately 40% of males seeking help at an infertility clinic with unknown etiology, idiopathic (Bracke et al., 2018; Ghuman and Ramalingam, 2018; Bala et al., 2021).

Recently, modifiable lifestyle plays a crucial role development of infertility, with generating growing interests to study such factors like frequent using of hot water, psychological stresses, aging, high temperature of scrotum, physical activity, using of mobile telephone, caffeine and nutrition (Hepworth, 2023; Ibitoye et al., 2023). However, relationship between life style stressor and quality of semen has provided evidences that semen quality is impaired by psychological stressors (Henriques et al., 2023).

In last decade, the clinical trials demonstrated that various antioxidants exerted a high efficacy in decreasing of sperm oxidative stress, and are summarized that supplementation of antioxidants in sub-fertile males improves outcomes of live births and pregnancy rates (Beygi et al., 2021; Torres-Arce et al., 2021). Different plant-derived pure molecules and herbal extract materials were revealed a high protection to several infections involving illnesses affecting the reproductive system (Alotaibi et al., 2021). Nigella sativa, belongs to dicot plant and classified under the *Ranunculaceae* family of *Ranales* Order, is one of the most historical famous ancient plants which well-researched for medicinal utilization among various countries (Salim et al., 2016; Rahim et al., 2023). In addition to N. sativa, there are several types of this plant which originate from one genus but they are differed in morphology and their chemical contents as *N. arvensis*, *N. orientalis*, and *N. damascena* (Benazzouz-Smail et al., 2023). The biological activity of N. sativa have been detailed by several researchers who evaluate the functional extracts,

essential oils, and fixed oil which used largely in nutraceuticals, pharmaceuticals and novel foods (Mukhtar et al., 2019; Silva et al., 2020; Balyan and Ali, 2022).
In Iraq, many authors have been investigated the activity of this palnt on bacteriological quality of soft white cheese (Alsawaf and Alnaemi, 2011), healing of different portions of extracted tooth sockets (Al-Hijazi et al., 2013), antimicrobial activity (Al-Shirifi et al., 2020), production performance and some immunological parameters in broiler chicken (Qader et al., 2020), and diabetic patients (Shari et al., 2020); while other studies target the effect of N. sativa in curing of infertility (Marbat et al., 2013; Ethaeb et al., 2021). Hence, this study investigates the role of N. sativa in enhancing the male fertility of mice through evaluation of serum testosterone, sperm motility, and sperm numbers and histology of epididymis and testes.

2. Methodology

Ethical approval
The College of Medicine (University of Al-Qadisiyah) licensed the present study.
Studyanimals
Totally, 30 male BALB/c mice of 6-8 weeks age old and 19-29 gm weight were purchased from the local markets, and acclimated for 1 week. During the acclimation and experimental periods, the study mice were fed pellet, received tap water and exposed to 12/12 dark / light.
Extraction
The seeds of N. sativa were purchased from the local market, sieved, grinded and dissolved in normal saline at a final concentration of 200 mg/ml
Experimental design
Initially, study mice were divided equally and randomly into two groups:

G1: Control group, the study mice were received 0.5 distilled water orally / day, for 28 days.

G2: Experimental group, the study mice were received the aqueous extract of N. sativa at a daily dose 300 mg/kg, orally for 28 days (Mohammad et al., 2009).

Samples
At the end of experiment, the study mice were euthanized with chloroform and the blood samples were drained directly into free-anticoagulant vacutainer tubes; while, tissue samples of epididymis and testes were collected carefully and kept into phosphate-buffered saline. Then, the caudal epididymis were divided into two parts; one to releasing the spermatozoa for sperm evaluation and the other kept into the 10% neutral buffered saline (NBF) for histology.
Serology
Following the manufacturer instructions (SunLong Biotech, China) of Mouse Testosterone ELISA Kit (Cat. No: SL0657Mo) Standards and sera were prepared, processed, and read at 450 nm. The concentrations of testosterone in sera were identified using the Standard Curve
Sperm examination
After surgical dissection of the caudal epidiymal tissue samples by the scissor and releasing of spermatozoa, the epididymal semen suspension (ESS) was incubated (37°C). Then, 5µl of ESS was placed on glass slide, and examined microscopically at magnifications of ×400 and ×1000 to detect the sperm motility (%) and number of sperms (×106), (NgahaNjila et al., 2019).
Histopathology
The epididymal and testicular tissue samples were kept initially into plastic containers contain 10% NBF; and then, they were dehydrated, cleared, infiltrated, embedded, sectioned (≈ 4-5 µm), and loaded on the slides. Post staining with Hematoxylin and Eosin, the slides were examined under the light microscope (MEIJI, Japan) at ×400 (Gharban et al., 2023).
Statistical analysis
The GraphPad Prism Software was served to identify significant differences between values of G1 and G2 groups at $P<0.05$ (*), (Gharban, 2022). Values were represented as Mean ± Standard Errors (M±SE).

3. Results and Discussion

Significantly, values of serum testosterone concentration measured in study mice were higher ($P<0.193$) in G2 (41.39 ± 1.51) than G1 (32.16 ± 1.38), (Figure 1). Also, the percentage of sperm motility were increased significantly ($P<0.0331$) in G2

(78.21 ± 3.48%) rather than of G1 (71.73 ± 4.17), (Figure 2). Concerning the number of sperms, slight significant elevation ($P<0.0491$) was observed in values of G2 (17.03 ± 1.27) when compared to those of G1 (16.44 ± 1.08), (Figure 3). Histologically, the findings of epididymal testicular tissues of G1 were showed a normal architecture; however, histological sections of G2 were enhanced remarkably ($P<0.05$) in testicular parenchyma and increasing in germinal cell population and spermatocytes. Similarity, the mature and immature Leydig cells numbers were also increased significantly with decreasing in degenerating cells number. However, significant alteration ($P>0.05$) in numbers of fibroblast and spermatogonia was not observed (Figures 4-7).

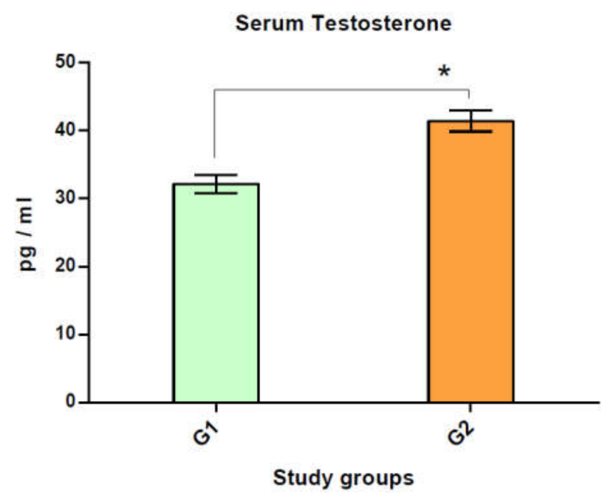


Figure 1 Results of serum testosterone in study mice of G1 and G2

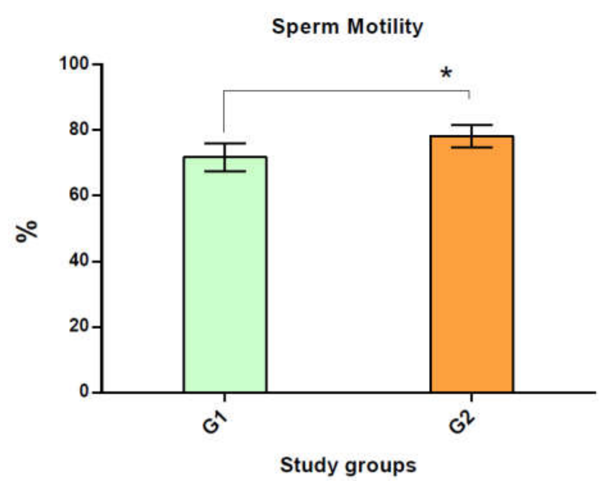


Figure 2 Results of sperm motility in study mice of G1 and G2

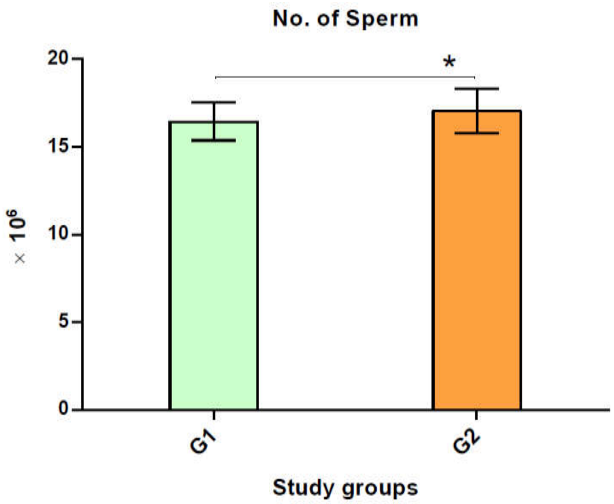


Figure 3 Results of sperm numbers in study mice of G1 and G2

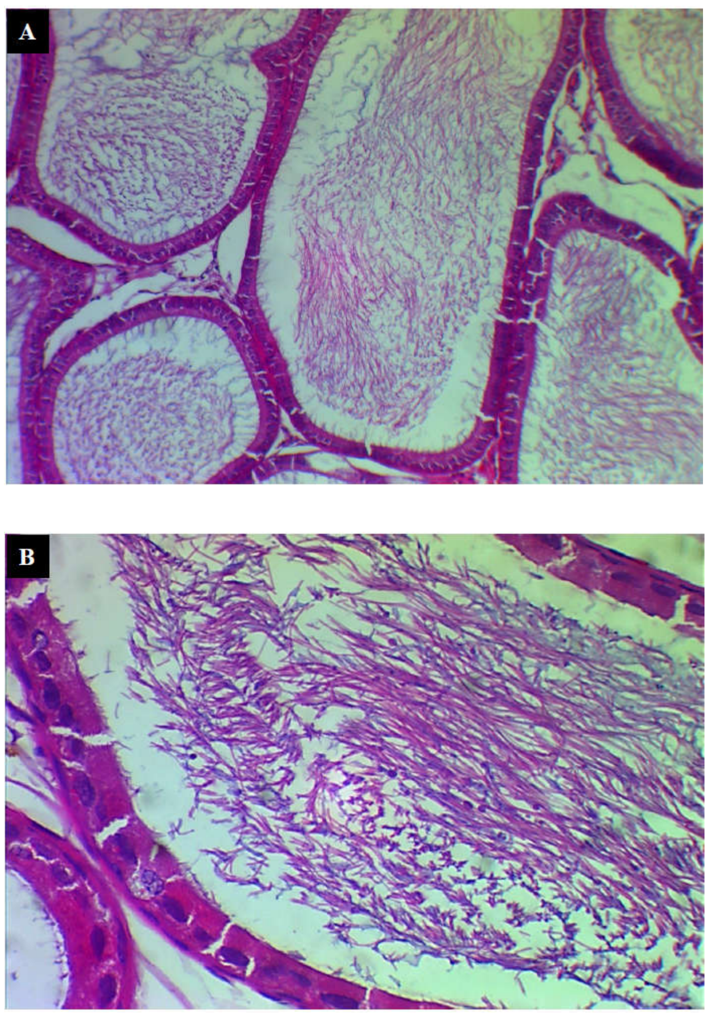


Figure 3 Histological section of epididymal tissue of G1 shows normal architecture; H & E; ×100 (A), and ×400 (B)

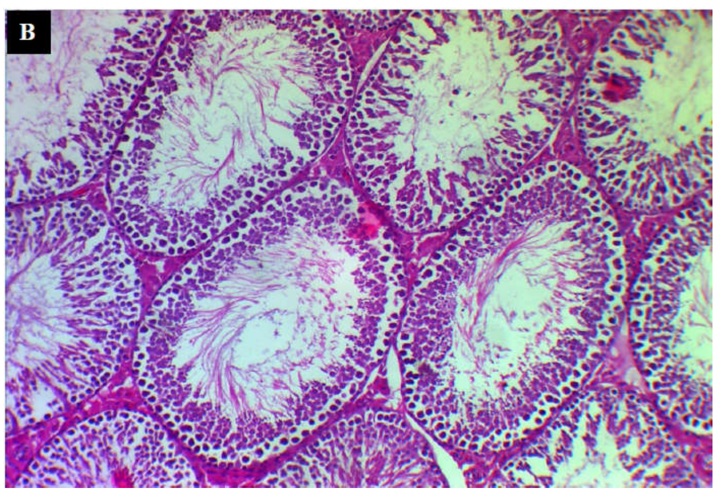
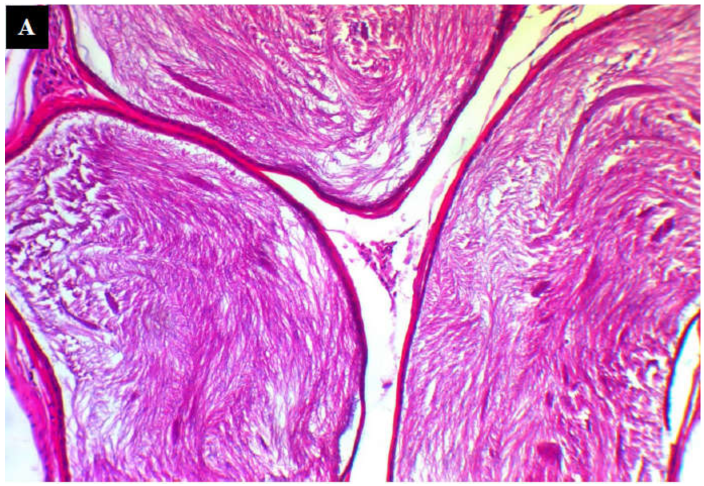


Figure 5 Histological section of testicular tissue of G1 mice shows normal architecture; H & E; ×100 (A), and ×400 (B)



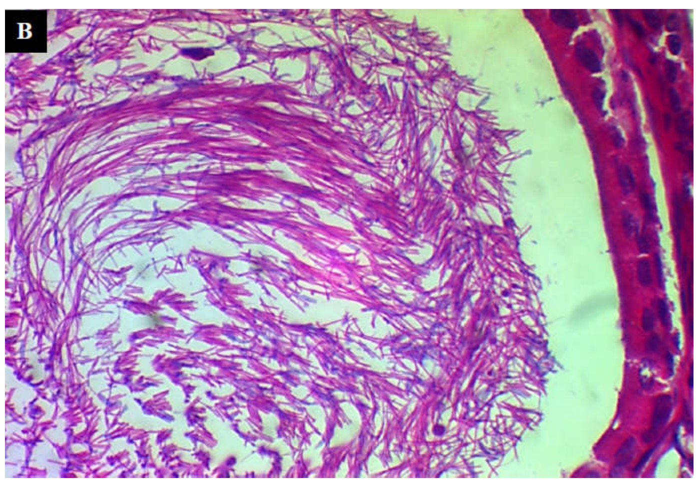


Figure 6 Histological section of epididymal tissue of G2 mice shows normal architecture; H & E; ×100 (A), and ×400 (B)

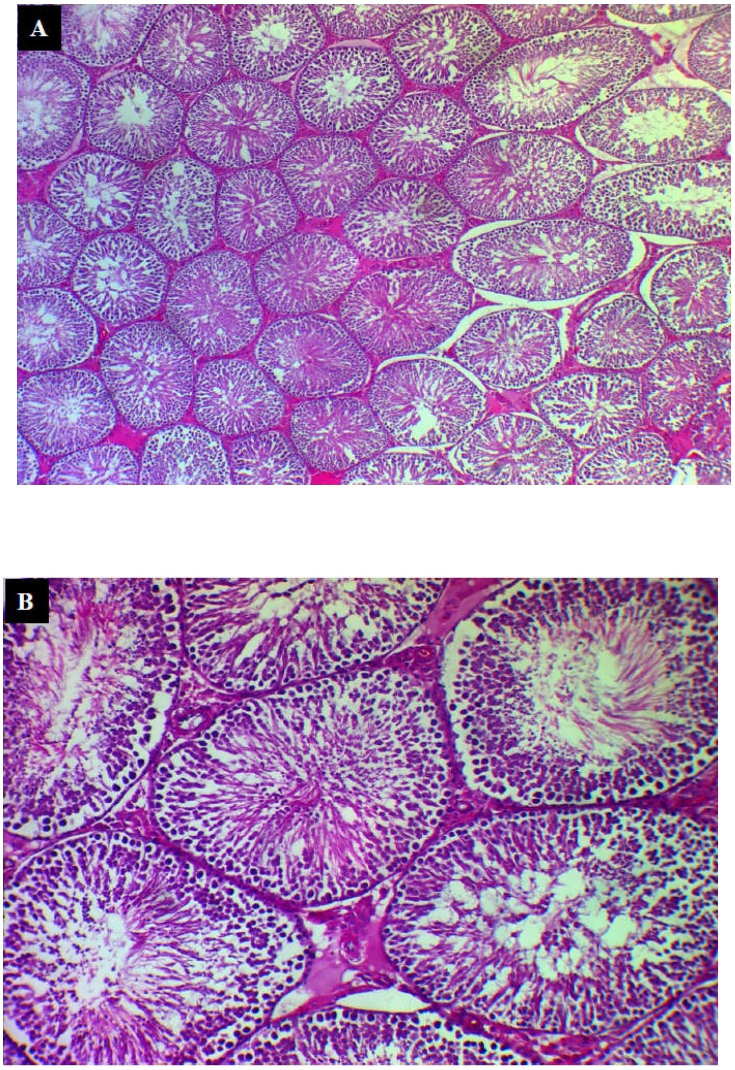


Figure 7 Histological section of testicular tissue of G2 mice shows normal architecture; H & E; ×100 (A), and ×400 (B)

The results presented in this work that show the positive effect of *N. sativa* on the level of serum testosterone, sperm motility and numbers of sperms were similar with those recorded by others (Rosli et al., 2019; Leisegang et al., 2021; Wani et al., 2022). This might be due to the role of *N. sativa* in stimulation either the activity of 17 β-hydroxysteroid dehydrogenase, the most important key enzyme in the testosterone biosynthesis pathway (Gromadzka-Ostrowska et al., 2002) or the secretion of sexual hormones that led to improve protein synthesis and decreases the serum cholesterol concentration in blood as reported by a previous study (Al-Taee, 2008). However, different results were recorded by various author; significant reduction in systolic blood pressure, waist circumference and body weight with insignificant reduction in diastolic blood pressure, fasting blood glucose and serum testosterone body (Datau et al., 2010); significant increasing in size of epididymis and testes, sperm count, fertility index, LH, and testosterone level with insignificant alteration in body weight and FSH (Parandin et al., 2012), significant increasing in sperm motility and weight of reproductive organs (Haseena et al., 2015), significant increases in serum testosterone concentration (Umar et al., 2017), significant improving the total body weight and testosterone in diabetic rats (Leisegang et al., 2021).

In a recent study, various components were detected in aqueous extract of *N. sativa* involving phenolic compounds, sesquiterpenes and terpenes (Zouirech et al., 2022). Various studies explore the consequences for administration of antioxidants to manage of male infertility (Adewoyin et al., 2017; Abarikwu et al., 2020; Abd-Elkareem et al., 2021). Phytochemical screening for secondary metabolites of *N. sativa* extract shows the presence of steroid, tannin, flavonides, coumarin, cardiac glycoside, saponin and diterpenes (Ishtiaq et al., 2013). Other observations indicated the presence of other chemical compositions as unsaturated fatty acids as linoleic, oleic, palmitic and stearic acids which actively improved the functions of reproductive system (Saxena et al., 2017; Mazaheri et al., 2019; Bayati et al., 2020).
Other researchers demonstrated a role of *N. sativa* in increasing spermatogenesis at primary and secondary spermatocytes and increase the weight of seminal vesicles (Mahmoud et al., 2002; Al-Sa'aidi et al., 2009). Other researchers recorded the increasing wall thickness and diameter of testicular seminiferous tubules account of spermatogonia (Tawfeek et al., 2006; Arak and Assi, 2011). Mohammad et al. (2009) confirmed that *N. sativa* supported testicular and epidiymal weight, seminal vesicle, ventral prostate and vase deferens but not the body weight that did not changed significantly. Also, the last authors were reported an increasing the population of germinal cells with decreasing of spermatocytes and spermatids. Similar findings recorded the increasing thickness of germinal layer but not epithelial layer of seminiferous tubules (Al-Zuhairy, 2012). Parandin et al. (2012) reported that testosterone can stimulate growing and secretary activities of reproductive organs, which reflect in increasing the function of somatic and germinal cells to allow for increasing of testicular and epididymal weights. In addition, thymoquinone, a major active component derived from *N. sativa*, is conformed to enhancing testicular parameters since it possesses a potent antioxidant activity that protect organs from oxidative damage by free radicals generating agents (Cho Ping et al., 2014). (Umar et al., 2017) found that the extract increases significantly the number of spermatogenic layers of testes, diameter of lumen of seminiferous tubules, diameter and area of seminiferous tubules, and thickness of spermatogenic epithelium.

4. Conclusion

The increasing level of serum testosterone and the histometry of epididymal and testicular tissues confirmed that aqueous extract of *N. sativa* stimulates releasing of androgen; whereas, the increasing sperm motility and numbers reflects the activity effect of extract on enzyme of oxidative phosphorylation. This confirms that the extract of this plant can be used as aphrodisiac and fertility promoting agent in both healthy and diseased individuals. Male infertility or sub-fertility occurs together with abnormal semen; therefore, enhancement of semen quality and quantity can be done with lowered advantages using the *N. sativa*. Moreover studies concerned the long term utilization of *N. sativa* and its effect on male and female reproductive system appear necessary to monitor the toxic potential of various xenobiotics.

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