

## A Comparative Study of the Effect of Mint Leaf Extract and Silver Nanoparticle on the Male Rats Treated with Ethidium Bromide

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### ABSTRACT

The present study was conducted for the purpose of verifying the effect of silver nanomaterial and comparing it with mint leaf extract on male rats treated with ethidium bromide. Where 50 male rats were selected and divided into 5 groups as follows: Control group 10 males rats gavage distilled water for 30 days, Ethidium bromide group 10 males rats gavage distilled water for 30 days, Ethidium bromide and mint leaf extract(40g/L) group 10 males rats for 30 days, Ethidium bromide and silver nanoparticle group 10 males rats for 30 days, Ethidium bromide and (silver nanoparticle, mint leaf extract) group 10 males rats for 30 days. And after sacrificed males rats we collected tissues samples from testis to extraction of RNA to evaluated of LH,FSH and testosterone by qPCR technique and tissues to make tissues section to knowledge changes in testis. The results of the current study, which was conducted on male rats, showed that there is a positive effect of mint leaves extract as well as a negative effect of silver nanoparticles on the level of gene expression for all study groups in each of the LH hormone, FSH hormone and testosterone compared to the control group as shown in the Fig 1,2 and 3. And showed in figure 4, several normal seminiferous tubules with normal spermatogonia cell, normal Sertoli cells, normal germ cell layer with normal maturation stages; interstitial spaces show an area of interstitial congestion; the lumen devoid of spermatozoa with interstitial congestion; normal seminiferous tubules with normal spermatogonia cell, normal Sertoli cells, and normal germ cell layer; maturation arrest of tubules and mild interstitial congestion; seminiferous tubules with atrophy exhibiting thick double cell layers indicative of cessation of spermatogenesis.

**KEY WORDS:** MINT LEAVE, SILVER NANOPARTICLE, LH, FSH AND TESTOSTERONE.

### INTRODUCTION

The Lamiaceae family is one of the families of flowering plants (Naghbi et al 2005) and genus *Mentha*, an important member of this family, has 6 species in the flora. These species have great importance in folk medicine and are available in traditional medicinal plant stores and local markets (Amin 2005). Spearmint is one of these species, with a good flavor and fragrance, used worldwide in pharmaceutical preparations, confectionery

and food industries, and also in hygiene and cosmetic products (Kumar et al 2005 and Spirling and Daniels 2001). Some studies have been shown that spearmint oil has anti-fungal, anti-microbial, anti-inflammatory, anti-tumor and antioxidant activity (Guimaraes et al 2011 and Pearson et al 2012).

Furthermore, various beneficial medicinal effects of spearmint have been found, such as preventing chemotherapy-induced nausea and vomiting (CINV), treatment of respiratory and digestive system disorders, hypertension, anxiety and even for relieving menstrual pain (Cakilcioglu et al 2011 and Tayarani-Najaran et al 2013). Spearmint is mainly recommended for its antispasmodic effects, which are related to its carvone content, the most important constituent of spearmint (29–74%). Spearmint also contains 4–24% limonene, 0.21–2.1% volatile oil and 3–18% cineole (Akdogan et al 2007). Nanoparticles (NPs) are particles that are designed

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and produced with a dimension or size that is  $\leq 100$  nanometers (Fathi, et al 2019).

Fertility, reproduction, and fetal development are essential to the sustenance of a species, highlighting the importance of the growing public awareness of the toxicity of NPs on the reproductive system. Recent studies have indicated an increased incidence of male reproductive defects, including low sperm production in adulthood, hypospadias, cryptorchidism, and testicular cancer (Pothuraju, et al 2013). This increased incidence of male reproductive defects may be partly attributable to environmental contaminant exposure. Thus, the wide use and environmental persistence of NPs have raised concerns about the negative impact on human health, especially on the reproductive systems and fetal health (Talebi, et al 2013). Nanoparticles have different effects on sperm cell functions either upon direct exposure under *in vitro* conditions or if administered *in vivo* (Shittu et al 2018).

The ability of NP to cross the hemato-testicular barrier has been demonstrated raising concerns about their distribution and biocompatibility at the systemic level. Several studies have reported the effects of Ag-NPs on epididymis, testis, and sperm function parameters in different animal species. However, the effects of NPs on oxidative stress and spermatogenesis need to be investigated on a case-by-case basis due to the administered concentration, and duration of treatment (Adenubi et al 2010).

## MATERIAL AND METHODS

### Study design

Fifty male rats weights ( $180 \pm 10$  gm), aged 90 days were selected and divided into 5 groups as follows:

1. C: Control group 10 males rats gavage distilled water for 30 days.
2. T1: Ethidium bromide (10 mg/kg b.w.) group 10 males rats gavage distilled water for 30 days.
3. T2: Ethidium bromide and mint leaf extract (40 mg/kg b.w.) group 10 males rats for 30 days.
4. T3: Ethidium bromide and silver nanoparticle (40 mg/kg b.w.) group 10 males rats for 30 days.
5. T4: Ethidium bromide and (silver nanoparticle, mint leaf extract) group 10 males rats for 30 days.

And after sacrificed males rats we collected tissues samples from testis to extraction of RNA to evaluate LH, FSH and testosterone by qPCR technique and some tissues to make tissues section to knowledge changes in testis.

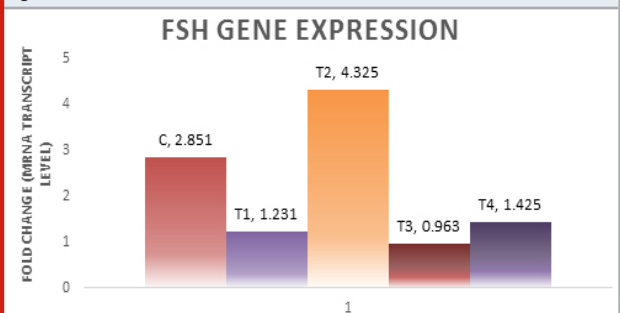
The method of induction by ethidium bromide: Gavage of ethidium bromide of experimental group of rats was performed with stomach tube. Ethidium bromide was used as in the form of red powder from Merck Company and saline was used as solvent.

**Mint leaf extract preparation method:** To prepare the mint leaf extract, a number of fresh mint leaves were prepared. The leaves were washed and their gel was removed. The gel was placed in 95 degrees ethanol with four times of their volume. After placing the gel container on the shaker for four days, the solution was filtered by a flat filter and concentrated using the rotary evaporator device at  $45^\circ\text{C}$ . The final material was completely dried at  $40^\circ\text{C}$  and powdered. Silver nanoparticles were acquired from Nanoshel nanotechnology organization in the US of America.

## RESULTS AND DISCUSSION

The results of the current study, which was conducted on male rats, showed that there is a positive effect of mint leaves extract as well as a negative effect of silver nanoparticles on the level of gene expression for all study groups in each of the LH hormone, FSH hormone and testosterone compared to the control group as shown in the Fig 1, 2 and 3. And showed in figure 4, several normal seminiferous tubules with normal spermatogonia cell, normal Sertoli cells, normal germ cell layer with normal maturation stages; interstitial spaces show an area of interstitial congestion; the lumen devoid of spermatozoa with interstitial congestion; normal seminiferous tubules with normal spermatogonia cell, normal Sertoli cells, and normal germ cell layer; maturation arrest of tubules and mild interstitial congestion; seminiferous tubules with atrophy exhibiting thick double cell layers indicative of cessation of spermatogenesis.

Figure 1: Fold change (mRNA transcript level) of FSH gene expression in male rats. No. 10 males per each. Different letters represent significant difference between groups ( $p < 0.05$ ).



Mint is a herbal plant which is usually recommended for treatment of many diseases, particularly digestive system problems; however, some studies show that despite its beneficial effects, mint has adverse effects on the reproductive system of male rats. Therefore, this study was designed to investigate the effects of mint leaves extract on the male reproductive system and fertility outcome. For successful fertility, normal structure and accurate function of all parts of reproductive system is needed. A complex mechanism under the regulated function of the hypothalamic-pituitary-gonadal axis (HPG axis) is responsible for initiation and maintenance of spermatogenic activity. Initially, by secretion of

GnRH (gonadotropin-releasing hormone) from the hypothalamus, FSH and LH are released from the pituitary gland. In the testes, under the stimulatory action of LH, the Leydig cells, located in interstitial tissue, produce and secrete testosterone.

Figure 2: Fold change (mRNA transcript level) of LH gene expression in male rats.

No. 10 males per each. Different letters represent significant difference between groups( $p < 0.05$ ).

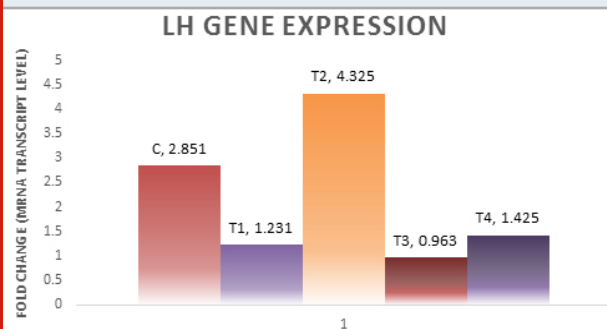
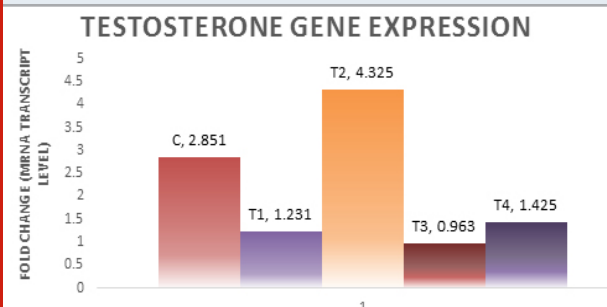


Figure 3: Fold change (mRNA transcript level) of testosterone gene expression in male rats.

No. 10 males per each. Different letters represent significant difference between groups( $p < 0.05$ ).

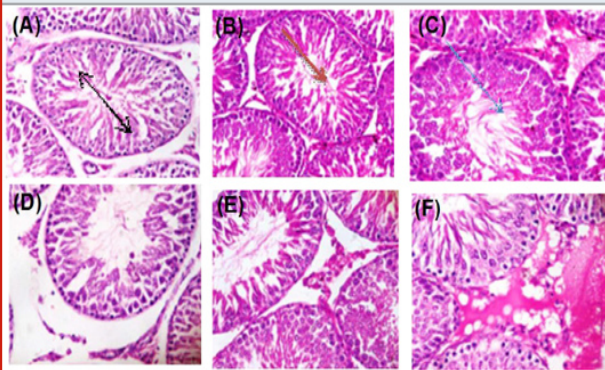


Simultaneously, FSH supports the function of sertoli cells, a mediator for effects of testosterone and FSH on germ cells for successful spermatogenesis in seminiferous tubules. After that, produced sperm pass through the epididymis, which secretes substances for sperm maturation. Ultimately, through the secretion of substances such as fructose, citrate, inositol and prostaglandins from seminal vesicles and secretion of prostatic liquid from the prostate, semen is produced. Thus any pathological changes in male reproductive tissues may interfere with fertility by altering the level of testosterone hormone or disturbing the spermatogenesis and sperm maturation process. In this study, results in normal testosterone level and also sperm concentration and motility, two critical parameters for male fertility (Nieschlag SMA, Nieschlag Behre 2020).

For evaluation of the fertility outcome of male rats, we also determined the number of their offspring born from mating of animals of all 4 groups with untreated females. Since mint administration did not cause any decline in number and motility of sperms, no difference was seen

in offspring numbers of experimental male rats compared to control. Moreover, the weight and crown-rump length of offspring were not affected. Therefore, we concluded that treatment of male rats with the mentioned doses of mint extract has no pathological, antiandrogenic or antifertility effects. This result is in contrast with the results of some investigations, which showed that mint has an antiandrogenic effect in male rats (Hafez and Hafez 2005).

Figure 4: Photomicrograph of testes sections after exposure of rats to mint leaves extract and Ag-NPs.



In their studies they used higher doses of mint extract and treated animals in a different way. They steeped mint tea (dried leaves) in a cup of boiling water and added it to the drinking water. So animals received mint continuously at all times for maximum 35 days, while we administered mint in lower doses, once a day for a longer time (45 days). Kumar observed no significant damage to the reproductive system following the short-term use of mint, but long-term use caused irreversible damage to this system, such as significant decrease in the weights of seminal vesicle, epididymis, testis and prostate with significant histopathological changes in these tissues. Also the level of LH and FSH decreased,

which was attributed to generation of oxidative stress in the hypothalamus and pituitary gland, leading to reduced production of GnRH and gonadotropins and resulting in an attenuated level of testosterone and spermatogenesis arrest in treated rats. In addition, severe histopathologic changes were observed in testicular tissue. Similar results were obtained in the study of Akdogan with the same doses and same duration of experiment. But higher levels of LH and FSH were observed in experimental animals followed by decreases in testosterone level and deficiency of spermatogenesis. They explained that an increase in FSH and LH is a normal process which occurs as the result of a decrease in plasma total testosterone levels and concluded that the deficiency of spermatogenesis is the consequence of the direct effect of mint on testicular tissue and Leydig cell dysfunction.

Studies have shown that changes in relative organs and body weight may be a sensitive indicator of the adverse effects of drug/chemicals or toxicants (Shittu et al 2015 and Berinyuy; et al 2015). The dose-dependent

decreases in all sperm velocity parameters, sperm total motility, and progressive motility, and the increases in sperm non-progressive motility, immobility, and sperm kinematics parameters following treatment with Ag-NP, compares well with those of Abu et al. 2013, Madan 2013, where low testosterone levels in animals impaired spermatogenesis by causing a decrease in sperm count and motility, increase in the percentage of defective sperm cells, and altered histomorphology of testis and/or epididymis.

The significant ( $p < 0.05$ ) dosed dependent decreases in the hormonal concentrations of LH, FSH, and testosterone in rats dosed Ag-NP for 7 days and 28 days were consistent with previous studies on the effect of different nanoparticles on hormonal levels of male rats. Adebayo et al. 2018 also reported a decrease in testosterone, FSH, LH, and prolactin after treatment with CeO<sub>2</sub>-NP. However, the present study contradicts the findings of Mathias et al. 2015 who reported that Ag-NP administered to rats at doses of 15 and 30 mg/kg; did not alter the hormonal levels of testosterone, estradiol, FSH, and LH profiles in rats. The low levels of LH and FSH might have hampered the secretion of testosterone from the testis with a consequent negative effect on steroidogenesis and spermatogenesis.

The generation of free radicals and induction of oxidative stress are the well-documented cellular effect of Ag-NP. Ag-NP penetrate the cellular organs particularly the mitochondria which impairs the membrane potential and induces the production of free radicals, this is evident by the elevated levels of H<sub>2</sub>O<sub>2</sub> and MDA, and the reduction of the activities of antioxidant enzymes, especially catalase, SOD, and GSH observed in testes and epididymis of rats dosed Ag-NP for 7–28 days. The decreased levels of catalase, SOD, and GSH after exposure to silver nanoparticles may be due to complexing of silver nanoparticles with thiol groups or to the increasing use of GSH, catalase, and SOD to downplay the effect of free radicals after exposure to of the nanoparticles.

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