American Journal of Biodiversity
https://biojournals.us/index.php/AJB
ISSN: 2997-3600

Potential Restoration of Spermatogenesis in Gentamycin-Compromised Testis throughout the Administration of Green Tea Supplement

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Received: 2024, 15, Sep
Accepted: 2024, 21, Sep
Published: 2024, 02, Oct

Annotation: This study aimed to evaluate the capability of the green tea leaf extract to decrease harmful effects on hormonal and histological changes in testicular tissue. The experiment was carried out on 24 Wister albino male rats, which divided into four groups (6 rats each group) and treated as following: first group was received normal saline (0.9 NaCl) to serve as control, the second group was injected with gentamicin (100 mg/kg, i. p), the third group was injected with gentamicin at the same doses and treated with the extract of green tea (200 mg/kg), and the fourth group was received extract of green tea (200 mg/kg) only. The drug of induction and all the treatments were given for 10 days.

The results showed a significant decrease ($p<0.05$) in the level of testosterone in the gentamicin group compared with the control

group and other groups. The group of green tea extract (200 mg/kg) and the (gentamicin +green tea 200 mg/kg) group did not show any significant difference ($p<0.05$) compared to the control group. The outcomes also demonstrated that using green tea extract reduced the histological damage caused by gentamycin to testicular tissue while raising testosterone levels.

In addition, the thickness of the interstitial tissue and the quantity of Leydig cells increased.

This suggests that green tea may have a function in tissue regeneration in addition to tissue repair, which could lead to several exciting applications for green tea in the protective effects of green tea extract on testis.

Keywords: Spermatogenesis, testosterone, green tea extract, gentamycin, male rats.

Introduction:

Green tea has been cultivated for centuries, beginning in India and China. Today, tea is the most widely consumed beverage in the world, second only to water. Hundreds of millions of people drink tea, and studies suggest that green tea (*Camellia sinesis*), in particular, has many health benefits (Cabrera *et al*., 2006).

Tea is believed to contain around 4,000 bioactive chemicals, a third of which are polyphenols. The active ingredients in green tea are powerful antioxidants called polyphenols (Tariq *et al*., 2010). Flavonoids (and their fraction, catechin) are the basic phenolic chemicals in green tea that are important for antioxidant actions, such as neutralizing free radicals produced during metabolism (Horzic *et al*., 2009).

Antioxidants are substances that fight free radicals, damaging compounds in the body that change cells, damage DNA, and even cause cell death (Venables *et al.*, 2008). Many scientists believe that free radicals contribute to the aging process, as well as the development of some health problems, including cancer and heart disease (Zhang *et al*., 2009). Antioxidants, such as polyphenols in green tea, can neutralize free radicals and may reduce or even help prevent some of the damage they cause (Seeram, 2006).

Various studies have shown that green tea extract has reproductive effects, improving some reproductive parameters. On the other hand, few studies have indicated a harmful effect of green tea extract on reproductive indicators. Therefore, future collaborative studies are needed to elucidate the optimal doses that will provide therapeutic benefits (Idowu, 2017).

Testosterone is the primary male sex hormone and an anabolic steroid. In male humans, testosterone plays a key role in the development of male reproductive tissues such as the testis and prostate, as well as promoting secondary sexual characteristics such as increased muscle and bone mass and the growth of body hair (Forest et al., 1973; Gann et al., 1996; Sheffield-Moore, 2000). In addition, testosterone is involved in health, well-being, and osteoporosis prevention (Raggatt and Partridge, 2010; Barrett and Ganong, 2012). Insufficient levels of testosterone in men may lead to abnormalities, including frailty and bone loss. (Fink et al., 2006). Low testosterone's

indirect effect on fertility involves a reduced sex drive that can result in a lack of desire even to have sex often enough for reproduction. (Berger et al., 2016; Corona & Maggi, 2022).

Gentamicin, this medication is used to prevent or treat a wide variety of bacterial infections. It belongs to a class of drugs known as aminoglycoside antibiotics (Whelton and Neu, 1982). It works by stopping the growth of bacteria sold under brand names. Garamycin, among others, is an antibiotic used to treat several bacterial infections (Hathorn et al., 2014). This may include bone infections, endocarditis, pelvic inflammatory disease (Dao et al., 2018), meningitis, pneumonia, urinary tract infections, and sepsis (Wilson, 2014; Garrett et al.,2000). Previous studies have demonstrated that gentamicin (100 mg/kg for ten days) can impair sperm motility, reduce reproductive organ weights, and cause apoptosis in the rat testes, eventually resulting in testicular failure (Aly, 2019).

The present study is designed to determine the effect of green tea extract on male rats treated with gentamycin. The parameters used included measuring testosterone levels and histological changes in male rats' tests that reflect the test's function or activity.

Methodology

Chemicals

Green tea leaves are collected from trees in Baghdad Governorate, sodium chloride (NaCl), 99% from Sigma-Aldrich, and gentamycin. Distilled water was used to prepare all aqueous solutions with no further purification, All the chemicals used in this study were of the highest purity.

Green tea leaves extract preparation

In this study, fresh green tea leaves were collected and washed with tap water and distilled water to remove existing dirt and dust. It was then left to dry naturally. After that, the leaves were ground using a blender (Phillips HR2096/00) so that their size became smaller than 0.5 mm. Finally, keep the resulting powder for further experiments.

Animal lab husbandry

All experimental animals were taken from the laboratory animals of the biology department in the science college of Thi-Qar University. Rats were kept in special plastic cages containing clean beds. Rats were housed under slandered laboratory conditions including a natural light cycle: 12 hours light and 12 hours darkness, room temperature ranged between (22± 2), and they were fed with a special diet in free form. It used 24 male rats (Rattus Norvegius) in age (10–12) weeks, and their weight ranged from (200 – 220) grams. Rats were equally divided into four groups (6 rats in each group):

The first group was treated with 0.5 ml normal saline 0.9 NaCl % for each animal; the second group was intraperitoneally injected with 100 mg/kg gentamycin drug (Faris, 2017); the third group was intraperitoneally injected with 100 mg/kg gentamycin then given 200 mg /kg green tea extract orally (Faris, 2017), fourth group, was given 200 mg/kg green tea extract orally.

2-Green tea extract:

Green tea extraction was done by mixing 10 gm of green tea leaf powder with 200 ml of distilled water and leaving the suspension in the water bath with continuous shaking for 24 hours at 40C°. After that, the extract was filtered using medical cotton then filtered by filter paper No.1 . Put the filterate in cleaned sterilized Petri dishes, left to dry, and the extract was kept as a powder for the use date.

3 – Blood collection and biochemical tests:

Ten days after the end of the experiment, rats were examined after the anesthetic, and blood was taken using the cardiac puncture method. The blood samples were in special test tubes free from anticoagulant substances, and then serum was isolated using a centrifuge at a speed of 3500

cycles/minute for ten minutes. Hormonal tests, which include testosterone hormone levels by using (kit of Monobind, USA).

During postmortem examination of experimental laboratory animals. We took the parts of the testes to fix them with a 10% formalin solution for preserving histological; sections were prepared from testes according to the method of (Humason, 1972).

Statistical analysis:

Results were represented as mean ± stander deviation and statistical analysis in one-way ANOVA and L.S.D test, at level ($p<0.05$) by using the SPSS program.

Results:

1- Testosterone hormone:

Table (1) shows a significant decrease ($p<0.05$) in the level of testosterone in the gentamicin group compared with the control group and other groups. The group of green tea extract (200 mg/kg) and the (gentamicin + green tea 200 mg/kg) group did not show any significant difference ($p<0.05$) compared to the control group.

Table (1): The effect of green tea extract on the level of testosterone in male rats treated with gentamycin.

Groups	Testosterone (mg/l) (Mean ± S. D)
Control	61.16 ± 8.44 [b]
Gentamycin	44.33 ± 5.75 [a]
Gentamycin + Green tea 200 mg/kg	66.66 ± 5.59 [b]
Green tea 200 mg/kg	58.83 ± 7.60 [b]
LSD	8.71

✓ **Similar small letters above the means indicate the non-significant differences, while different letters indicate the significant differences.**

1- Histological examination:

The photomicrograph section of the testis is in a control rat that shows the normal structure of seminiferous tubules (arrow). Also, complete spermatogenic figures (double heads arrow) and obvious interstitial fibres with Leydic cells were observed (thin arrows). Seminiferous tubules lined with spermatogonia and primary spermatocytes (thick arrow); moreover, spermatids and sperms were clearly found within the seminiferous tubule lumen (star). H&E (10x), (figure 1).

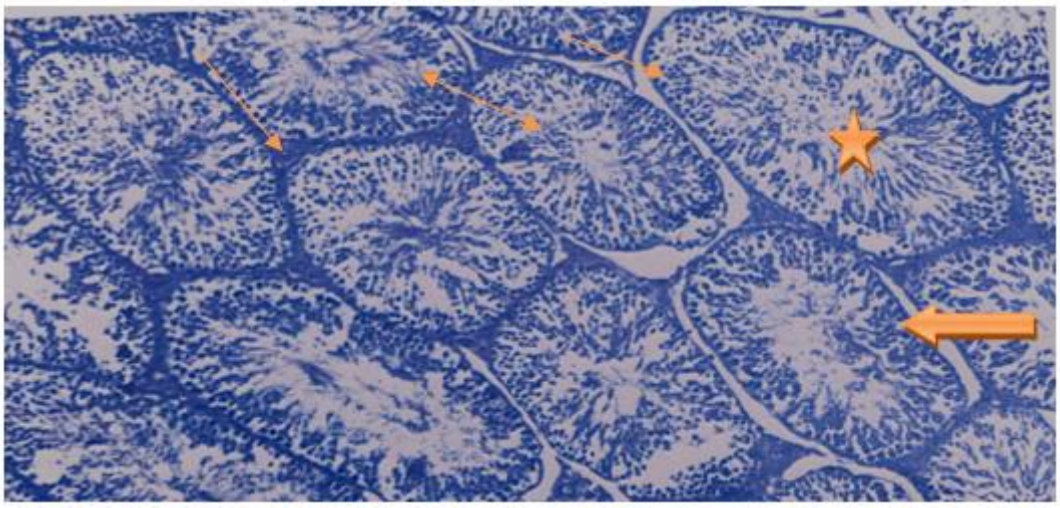

Figure 1: A photomicrograph of the testicle's typical tissue structure in the control group.

Morphological histology of testes in rats treated with gentamycin showed clear detachment of spermatogonia from the basement membrane and degeneration of spermatocytes with shrunken pyknotic nuclei lining seminiferous tubules (thick arrow), with loss of spermatids and sperms in tubular lumen (thin arrow). It also increases interstitial space with the degeneration of Leyding cells. H&E. 10X (figure 2).

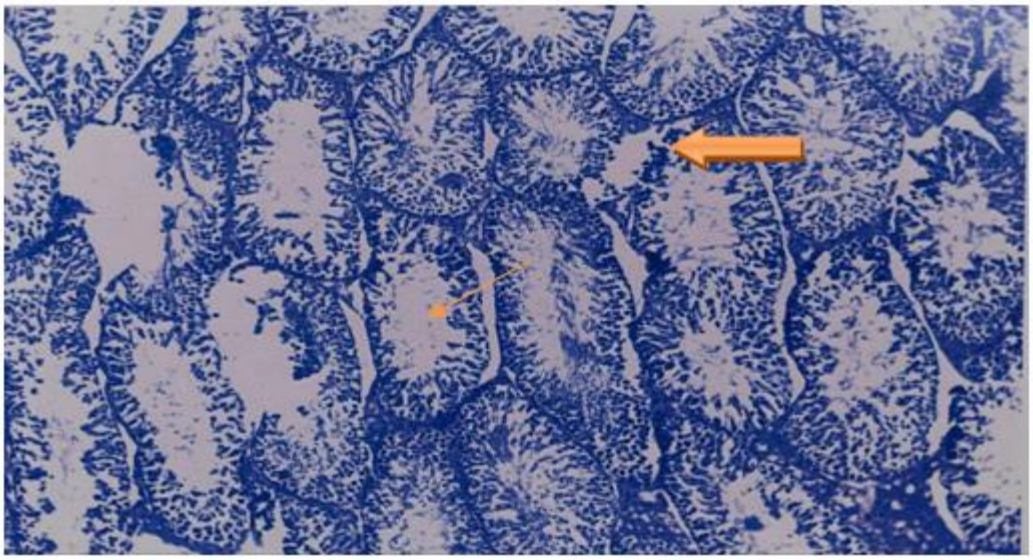

Figure 2: A photomicrograph of the testicle's abnormal tissue structure in the gentamycin group.

Testis section of rat-treated tea extract and gentamycin representing obvious rebuilding in seminiferous tubules throughout spermatogenic figures appeared (thin arrow), with increased intensity of spermatids and sperms within a tubular lumen (thick arrow), and some regenerated interstitial tissue (star arrow). Furthermore, it shows spermatogonia attached to the basal membrane of seminiferous tubules (double heads arrow), with regenerated interstitial tissues and clear Leyding cells (star). H&E (10), (figure 3).

Figure 3: A photomicrograph of the testicle's tissue structure in the gentamycin + green tea group.

The histological section of the testis in the group treated with green tea showing all structural architectures of testicular components were represented comparable to the control group, H&E.(10x) (figure 4).

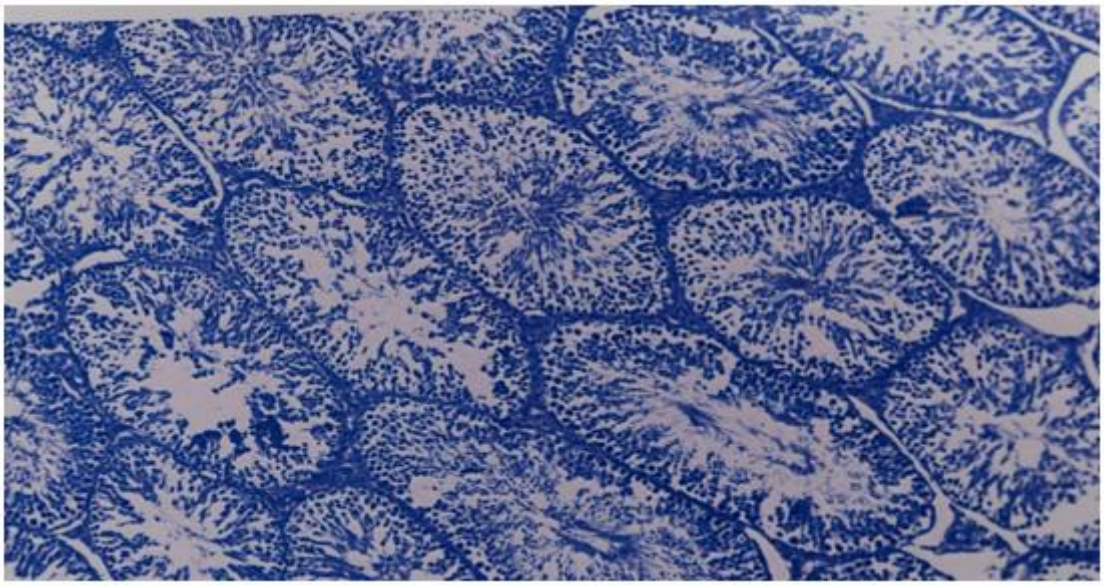

Figure 4: A photomicrograph of the testicle's tissue structure in the green tea group.

Discussion:

Several causes may affect male fertility, so the current study revealed that gentamycin-induced disturbances were present at both functional and structural levels. Meanwhile, our results indicated the protective effect of green tea extract restoring the level of testosterone and histological changes of testicular tissue. Gentamicin (G) is known to have harmful effects, such as an inhibitor of steroidogenic enzymes in the testicles (Ghosh *et al.*, 1999), as well as calcium-dependent activity Phosphatidylinositol phosphodiesterase (Shute and Smith, 1985).) and thus, it has been shown the inhibitory effect on spermatogenesis processing and level of testosterone. Moreover, it was found that corresponding to the former report explains the decrease in the level of testosterone reported in males with rheumatoid arthritis (Gordon *et al.*, 1986). On the other side, there were previous results consistent with our study results; Gentamicin documented a significant decrease in the level of testosterone compared to the control group (Carageorgiou *et al.*, 2005), which may attributed to the chronic use of calcium channel blockers, diltiazem, and cinnarizine caused a marked decrease in circulating blood levels of testosterone in male rats (Morad *et al.*, 1997) also Gentamicin is a cationic substance that interacts with Ca++ bound to the lipid monolayers and bio membranes (Vollmer, 1982).

Histological changes in the testicular tissue might attributed to gentamicin decreasing rat testis's weight and inhibiting spermatogenesis. It induces oxidative stress and apoptosis by possible mitochondrial dysfunction (Aly, 2019). Remarkable regeneration of spermatogenic figures within somniferous tubules due to the protective effect of green tea extract; moreover, it has antioxidant properties such as flavonoids, metals, and vitamins, which play an important role in the induction of cellular growth and prevention of free radicals induced oxidative stress (Rai-Nishant et al., 2012). Green tea polyphenols GrTPs act as free radical scavengers, protecting spermatozoa against oxidative stress (Chiuet al., 2016). Testicular tissue is predisposed to suffer from the action of free radicals and OS because of its high cell division rate, oxygen consumption rate, low oxygen pressure, debilitated vessels, and high levels of unsaturated fatty acids (Hatasa et al., 2016). They also revealed that GT has aromatase inhibitor activity, which could be the main cause of improved serum levels of testosterone (Sato et al., 2010). Lowering estradiol levels by administering an aromatase inhibitor is associated with increased levels of luteinizing hormone LH and testosterone (T'Sjoen et al., 2005]. Aromatase inhibitors, therefore, have been suggested as a tool to increase

testosterone levels in men with low testosterone levels (Kaufman and Vermeulen, 2005). Drinking green tea also helped reduce pathological histological alterations. El-Shahat et al. (2009) revealed a similar finding that green tea extract prevented the adverse effects of dimethoate on testicular tissue.

We infer from the present study that green tea extract, due to its high content of antioxidants, minerals, and vitamins, plays a key role in enhancing the inhibitory effects of gentamicin on testicular function while inhibiting the aromatase enzyme.

Conclusion

We conclude from the current study that green tea extract has a crutial role in improving the inhibitory effects of gentamicin on testicular function due to its content of important antioxidants, minerals and vitamins, Also by inhibiting the aromatase enzyme

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