Eliminating amoxicillin-induced testes toxicity by Ashwagandha seeds

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Abstract

The adverse effects of antibiotics are still a vital problem **in** human life. Authors in this work have examined the negative impacts of one of the most widely used drugs, amoxicillin (AM), on the testes of male rats, and also addressed how to deteriorate these effects by utilizing a natural product, Ashwagandha seeds extract (ASE) by comparing the role of the extract as a protective and therapeutic agent. Histopathological analysis of the testes showed that ASE could enable the biosystem to defend the testicular tissue against the toxic effects of AM. It proved that the protective use of ASE was better. Moreover, dielectric and thermodynamic parameters (electrical impedance, relaxation time, electrical conductivity, activation energy, and change in enthalpy) revealed their aptitude in discriminating the toxic AM and ameliorative ASE impacts. The medical action of ASE is due to its content of polyphenols and flavonoids. In other words, ASE proved its ability to help the biosystem repair some of the AM side effects on testes' tissues, and dielectric parameters proved their capability to detect toxicity and repair in testes tissues.

Keywords

Dielectric properties of testes; Histopathology; Impedance; Activation energy; Enthalpy.

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1. Introduction

It's well known that utilizing chemical drugs, especially common antibiotics, has antagonistic effects on the biological organs such as the liver, heart, kidney, brain, and reproductive system. In this work, the authors have focused on the testes as one of the sensitive organs that can be affected by an overdose of antibiotics and chose amoxicillin as one of the most commonly used antibiotics in the world.

A lot of studies investigated the effect of different antibiotics such as enrofloxacin, tetracycline, trimoxazole, amoxicillin, erythromycin, and chloroquine on testicular cells. Most of these antibiotics can cause remarked decrease in the testis weight, sperm motility, sperm count, and living spermatozoa cells count, as well as changes in sperm morphology, and generally in the testis histopathology [1-3]. Another work revealed the effect of amoxicillin-clavulanic acid with doses of 30 and 150 mg/kg/day causes a reduction of sperm motility [4]. Bouchicha et al. [5] studied in vitro sperm quality after administration of some antibiotics including Amoxicillin + clavulanic acid. They reported that the antibiotics have an inhibitory effect on sperm motility with linear speed changes that have an adverse effect on fertilization. A recent study investigated the impacts of oral amoxicillin administration during pregnancy at different times of pregnancy stages on fetal testicular growth with doses (75, 150, or 300 mg/kg· d). Their results displayed abnormality in the fetal testicular morphology, inhibition in the cell proliferation, apoptosis development, and decrement in the level of fetal blood testosterone [6].

One of the safe methods for fading the adverse effects of amoxicillin on testes is the administration of natural herbs. In our work, we have used Ashwagandha seeds to estimate their role in the improvement of testicular toxicity induced by amoxicillin overdose.

Previous studies revealed that stress can cause infertility and suggested that Ashwagandha roots can adverse the stress impact due to controlling the sexual hormone levels [7]. In addition, Shukla et al. [8] observed that the treatment of seminal plasma of infertile men with Ashwagandha roots led to a reduction in apoptosis and intracellular level of reactive oxygen species in spermatozoa and controlling the level of essential

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metal ions as Zn^{2+}, Cu^{2+}, Au^{2+}, and Fe^{2+}. Gupta et al. [9], also, noticed an improvement in the levels of lactate, citrate, and some aliphatic and aromatic amino acids in seminal fluid and recovery of the semen quality in the infertile men treated with Ashwagandha roots. Furthermore, Rahmati et al. [10] proved that Ashwagandha roots can increase sexual hormones' levels. Durg et al. [11] mentioned that the action mechanisms of Ashwagandha, *Withania somnifera,* and its active elements specialist in the treatment of male infertility are still unknown.

This work aims to estimate the role of Ashwagandha seeds' extract in calming the testicular toxicity persuaded by a toxic dose of amoxicillin. This has been investigated by histopathological analysis of testes, electrical impedance, relaxation time, electrical conductivity, activation energy, and enthalpy change.

Experimental

2.1 Amoxicillin:

The AM drug capsules were liquified forming a dose of 90 mg/kg. b. wt. in distilled water [12] and was administered orally to rats for twelve days once daily.

2.2 Plant material (Ashwagandha):

Ashwagandha seeds were obtained from The Horticultural Crops Technology Department in our institute. Ashwagandha seeds' Aqueous extract was made according to Maheswari and Manisha's method [13].

2.3 Experimental animals:

Sprague-Dawley male rats (about 120 g) were used in this study. Rats were bought from Animal House in our institute. The animals were housed in Stainless Steel wire mesh cages containing wood shavings as bedding. The lab's temperature was controlled to be 25 $\pm$ 3C with a relative humidity of 50% $\pm$ 15. Food and water were provided *ad libitum.*

2.4 Ethics:

The protocol of animals' care during experimental procedures was approved by the Medical Ethical Committee of our Institute, No. (05480723).

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2.5 Experimental design:

Fifty-six rats were used in the experiment. They were divided into eight groups of seven animals in each group. as follows:

Group	Treatment
Control (G1)	Healthy untreated rats
Amoxicillin (G2)	AM was Orally given at a dose of 90 mg/kg. b. wt. daily for 12 days
Protected groups with ASE (G3-5)	As G2, AM was Orally given at a dose of 90 mg/kg. b. wt. daily for 12 days. Two hrs. after the AM treatment starting from day one, the groups (G3-5) received, orally, ASE (100, 200, and 300 mg/kg) respectively for the same period of AM treatment.
Therapeutic groups with ASE (G6-8)	As G2, AM was Orally given at a dose of 90 mg/kg. b. wt. daily for 12 days. After 24 hrs from the last AM dose, groups (G6-8) were orally administrated with ASE (100 mg/kg, 200 mg/kg, and 300 mg/kg) respectively, for 7 days.

2.6 Histopathological examination:

After abdominal incision, the testes were collected and fixed in 10% formaldehyde solution for 24h, then samples were prepared in a specialized histopathological laboratory [14]. After that, the processed samples were checked under a light microscope.

2.7 Dielectric measurements:

2.7.1 Sample preparation:

The fixed testes samples in 10% formaldehyde solution, were cut into Cross-sectional parts and hydrated in distilled water for 24 hrs. Then samples were dehydrated in a series of concentrated ethyl alcohol concentrations of 50, 70, 90, 95, and 100 % where samples remained for 30 minute**s**

s in each concentration respectively before dielectric measurements were performed.

2.7.2 Dielectric measurements and thermodynamic state functions:

Dielectric measurements were done using a Broadband dielectric spectrometer (BDS), Concept 40, from Novo Control, Germany with a frequency range from 0.1 Hz to 20 MHz, and applied voltage 1 $V_{rms.}$ The measurement of samples was carried out using two parallel brass electrodes of 10 mm diameter for the small one. The temperature range

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(10- 40 C) was controlled automatically by the BDS system with a temperature accuracy of 0.1 C.

The dielectric response of testes tissue under the effect of a uniform electric field for all groups is measured. The equations of both fitting measured physical parameters and their related thermodynamic state functions were used according to the previous works [15-24].

a) Impedance:

$$Z^*(\omega,T) = \frac{R_{DC}(T)}{(1+(i\omega\tau\,(T))^{\alpha})^{\beta}} \qquad (1)$$

where Z^* is the complex impedance, R_{DC} is the direct current resistance, i is the $\sqrt{-1}$, ω is the angular frequency equals 2πν and the latter is frequency, T is the temperature, τ is the relaxation time. Shape parameters are defined as $0 < \alpha \leq 1$ and $0 < \beta \leq 1$.

b) Relaxation time:

$$\tau = \tau_o \, exp[-\frac{Ea}{RT}] \qquad (2)$$

where, τ is the relaxation time at any absolute temperature, T, τ_o is a pre-exponential factor, Ea is the activation energy, and R is gas constant.

c) DC conductivity:

$$\sigma = \sigma_o \, exp[-\frac{Ea}{RT}] \qquad (3)$$

where σ, direct current, dc, is conductivity at any absolute temperature, σ_o is a pre-exponential factor, Ea is the activation energy, and R is the gas constant.

d) Activation energy:

Activation energy is extracted from equations (2) and (3).

e) Enthalpy change, ΔH:

$$\Delta H = R.\frac{\partial(Ln\tau)}{\partial(\frac{1}{T})} - R \qquad (4)$$

where ΔH is the enthalpy change, and τ is the relaxation time of the specified process.

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1. Results

1.1 Histopathology of testes samples:

1.1.1 Protective ASE groups compared to control and AM:

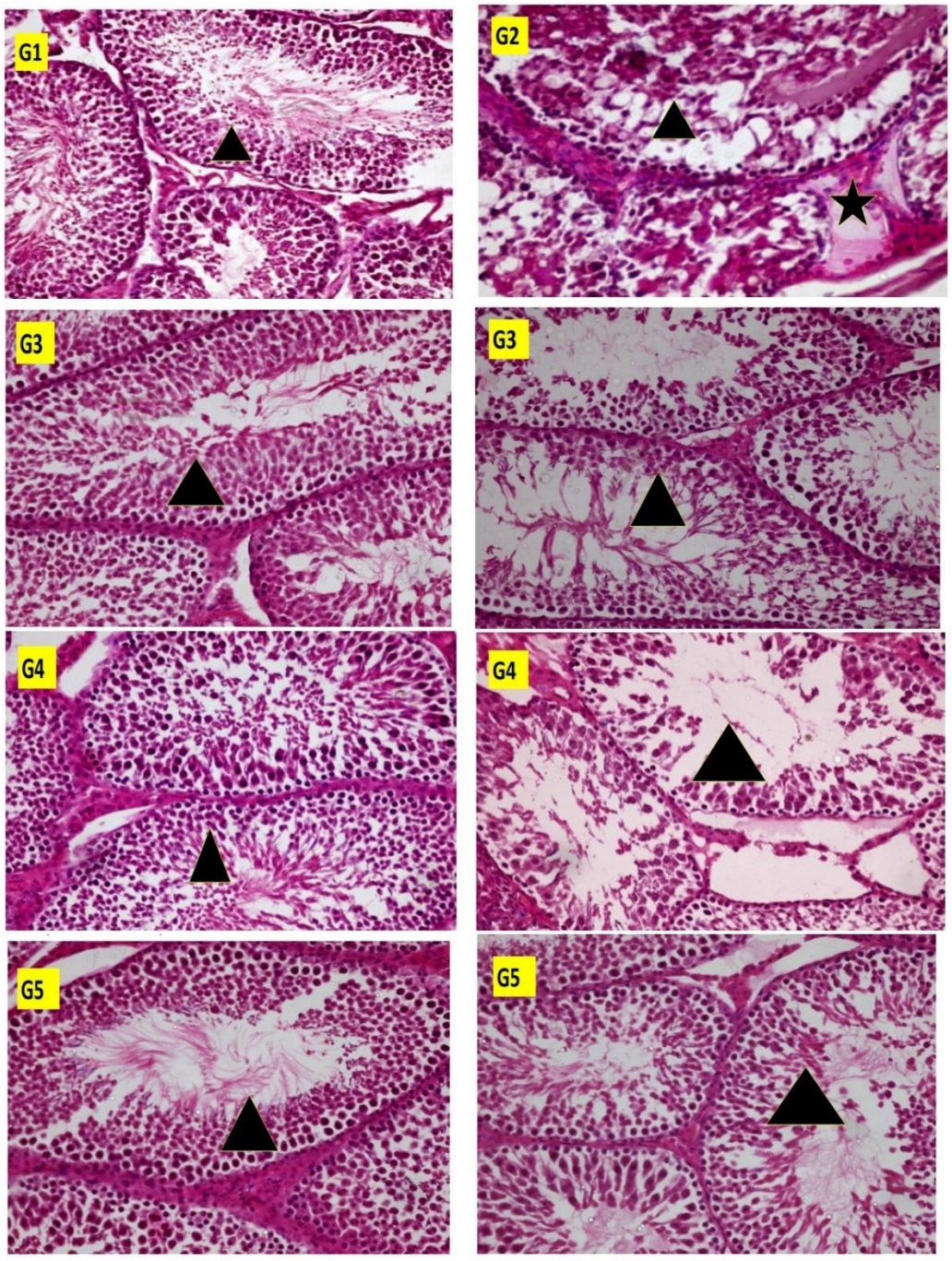

Fig. 1. Testes photomicrograph of (G3-G5) compared to G1 and G2.

G1, Control, shows normal structure of seminiferous tubules with normal spermatogenesis cells series (triangle) and normal Leydig cells in the interstitial tissues.

G2, Amoxicillin group, indicates spermatogenesis cells series depletion with vacuolar degenerative changes in its cells, disarranged epithelial layers (triangle), edema and congestion of blood vessels between seminiferous tubules (star).

G3, Protected group, 100 mg/kg of ASE, shows normal spermatogenesis cells in most of seminiferous tubules on the left side and mild degeneration of spermatogenesis cells series (triangle) on the right side with normal seminiferous tubules.

G4, Protected group, 200 mg/kg of ASE, displays normal spermatogenesis cells in most of seminiferous tubules and slight degeneration of spermatogenesis cells (triangle) in other tubules with intraepithelial empty spaces between the seminiferous tubules.

G5, Protected group, 300 mg/kg of ASE, shows spermatogenesis cells series with seminiferous tubules and Leydig cells nearly as control.

From the previous photomicrographs, Fig. 1, the normal testicular tissue has been shown in control group (G1) with spermatogenesis cells, seminiferous tubules, and Leydig cells in the interstitial tissues. Amoxicillin administration, G2, caused a severe depletion in the spermatogenesis cells with vacuolar degeneration, adding to disarranged epithelial layers, edema, and vascular congestion between seminiferous tubules. On the other side, the protected groups, G3-5, showed a noticeable improvement in the testicular tissue at all doses of ASE compared to AM group. The best case was observed at the highest dose, 300 mg/kg.

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1.1.2 Therapeutic ASE groups compared to control and AM:

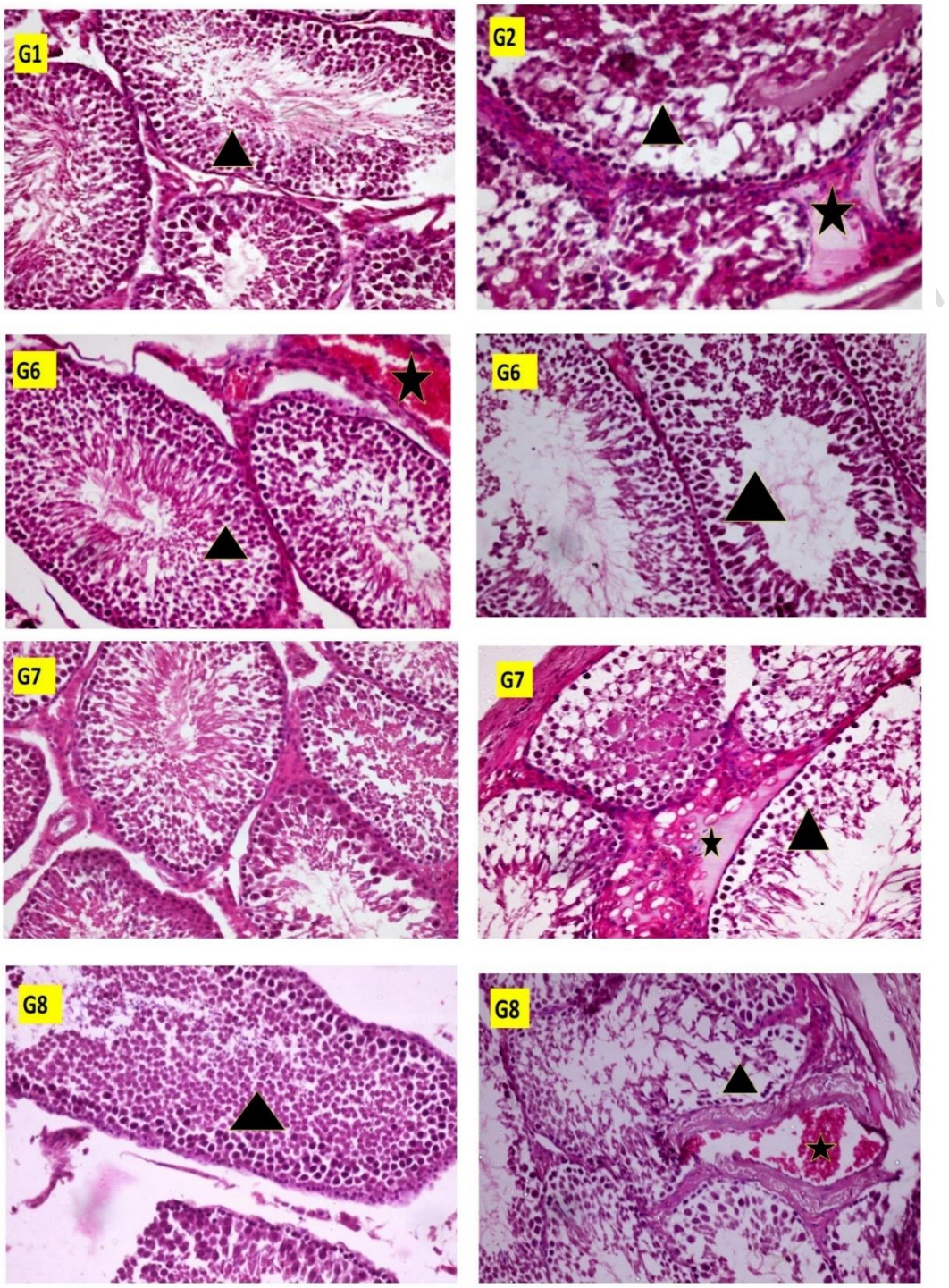

Fig. 2. Testes photomicrograph of (G6-G8) compared to G1 and G2.

G1, Control, shows normal structure of seminiferous tubules with normal spermatogenesis cells series (triangle) and normal Leydig cells in the interstitial tissues.

G2, Amoxicillin group, indicates severe spermatogenesis cells series depletion with vacuolar degenerative changes in its cells, disarranged epithelial layers (triangle), edema and vascular congestion between seminiferous tubules (star).

G6, therapeutic group, 100 mg/kg of ASE, demonstrates some seminiferous tubules with normal spermatogenesis cells and congested blood vessels and others suffer from moderate degeneration of spermatogenesis cells (triangle).

G7, therapeutic group, 200 mg/kg of ASE, shows some seminiferous tubules with normal spermatogenesis cells and others have an inhibition and vacuolar degeneration in the spermatogenesis cells (triangle), disarranged epithelial layers, edema and congestion of blood vessels between seminiferous tubules (star).

G8, therapeutic group, 300 mg/kg of ASE, reveals some seminiferous tubules contain immature spermatogenesis cells and others suffer from a severe depletion of spermatogenesis cells series, altered shape of seminiferous tubules, dilation and congestion in interstitial blood vessels.

In Fig. 2, the tissue improvement was remarked in the therapeutic ASE group (G6) with the lowest dose (100 mg/kg), compared to AM group (G2), in some seminiferous tubules, while the others had mild degeneration with vascular congestion in the interstitial tissues. At the middle dose of ASE, the testicular tissue was enhanced in some seminiferous tubules, and a vacuolar degeneration was observed in the others with edema and vascular congestion between seminiferous tubules. Additionally, at the highest dose, the growth of immature spermatogenesis cells was pronounced in some tubules, while the others had severe degeneration in the spermatogenesis cells series, disarranged epithelial layers, altered shape of seminiferous tubules, thickening, and congestion of interstitial blood vessels.

From the results of Fig. 1 and 2, it was noticed that the changes in the spermatogenesis cells, seminiferous tubules, and interstitial tissues in the protected groups were less than those in the therapeutic. Accordingly, ASE, as a protective agent, could defend the testicular tissue more than using it in the therapeutic case.

Several works confirmed the negative effects of some antibiotics, including amoxicillin, on sperm count, sperm motility, living spermatozoa cell count, and generally on the testicular tissue [1-5, 12]. Also, Elnasharty et al. [25] revealed sperm shape anomalies due to amoxicillin toxicity. Other studies showed that the overdose of AM can cause oxidative stress [26], and the presence of polyunsaturated fatty acids in the spermatozoa cells may be the reason for their sensitivity to the oxidative stress producing lipid peroxidation, which leads to testicular dysfunction [27-31].

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On the other hand, the protective role of ASE against the negative AM effects on the testes was observed significantly. Besides, the therapeutic role of ASE was vital in some testicular tissues and couldn't be effective in others. This indicates that the testes are sensitive to AM doses, therefore, the ASE administration with AM- protective mode- helped the testes to recover more speedily. In the therapeutic ASE case; ASE was given after the AM damages, already occurred which may require a longer period and/ or a prolonged ASE gavage time, more than seven days to repair the present tissue injuries. Generally, our results proved that the Ashwagandha seeds have a similar feature as Ashwagandha roots in fading the testicular toxicity. These data are confirmed by previous work [25] which revealed that protective and therapeutic ASE can minimize the sperm shape anomalies, resulting from amoxicillin toxicity, and the positive impact of protective ASE was more obvious.

However, the exact action mechanism of Ashwagandha on infertility is not known [11], but it was suggested that antioxidant administration can develop the sperm function, so the presence of several polyphenols and flavonoids in Ashwagandha can scavenge the free radicals that were indicated in the rats and mice testes due to AM treatment in previous studies. Moreover, the ability of Ashwagandha to control the reproductive hormone levels can help in the enhancement of sperm quality [25, 32-37].

1.2 Dielectric measurements:

3.2.1.1 Impedance relaxation of testes:

Fig. 3, shows, in the upper part, the imaginary part of testes impedance of control group (G1) in the temp. range (10-40C), while the lower part explores the fitting data of real and imaginary parts of testes impedance at 37C with frequency (0.1 Hz- 20 MHz).

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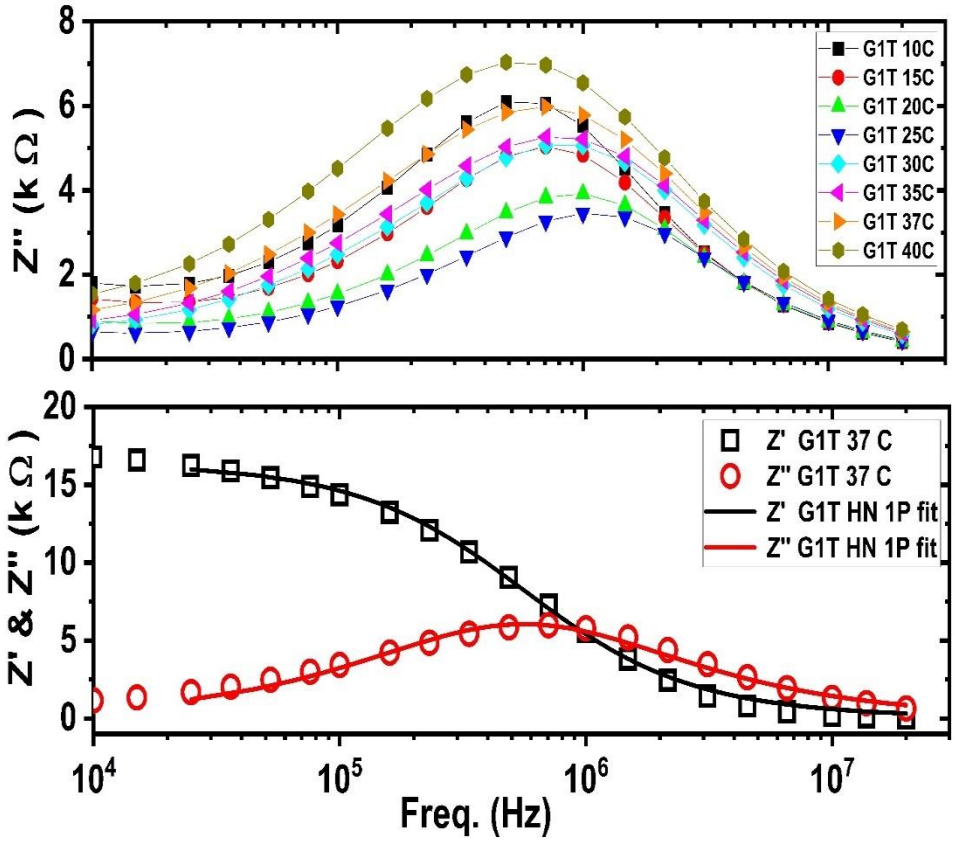

Fig. 3. Imaginary part of testes impedance of control, G1T, (10-40C) with frequency (0.1 Hz- 20 MHz) (upper Fig.), and fitting data at 37C (lower Fig.).

Impedance detects testes interaction with the electric field displaying a relaxation peak covering frequency range of 20 kHz- 20 MHz. Havrliak-Negami equation was used to fit the measured data and extract basic parameters, relaxation time, and DC-conductivity. The outcome of the fitting process is used to compare the AM and Ashwagandha effects on the testes and to assume other parameters (relaxation time, DC conductivity, enthalpy, and activation energy). These calculations were carried out for all groups (G1-G8) using the equations (2-4) [18-24].

3.2.1.2 Real and imaginary parts of testes impedance (G1-G8):

Fig. 4, shows the real part of testes impedance, Zs' (upper Fig.), and imaginary part, Zs" (lower Fig.) for all groups at 37C with frequency (0.1 Hz- 20 MHz).

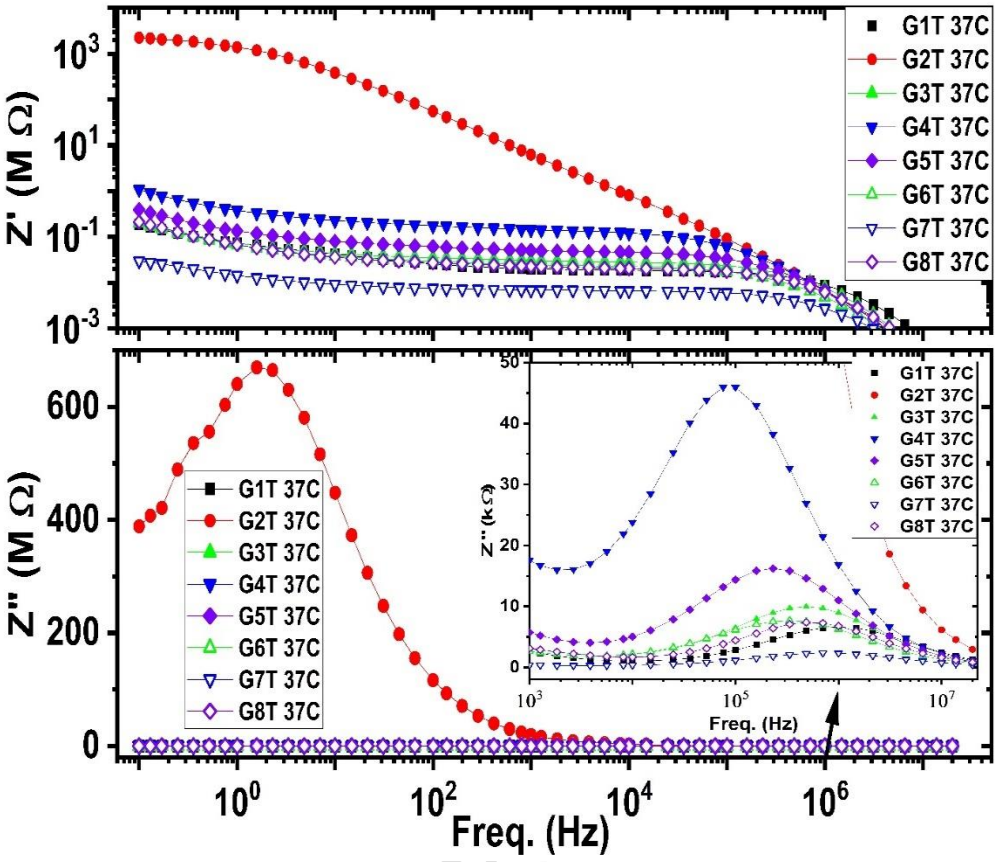

Fig. 4. Real part of testes impedance, Zs′ (upper Fig.) and imaginary part, Zs″ (lower Fig.) for all groups at 37C with frequency (0.1 Hz- 20 MHz).

It's noted that AM interactions made a gigantic increase in the impedance and a massive decline in the relaxation time of the testes' tissues. Both types of ASE uses were very successful in retaining both the impedance and relaxation time of the testes tissues to ranges near that of control. Where the therapeutic use of ASE was more successful. For both remedies, the intermediate dose of 200 mg/kg/day was the furthest from the control.

The observed relaxation peak of the imaginary part of impedance at about 1 MHz belongs to the orientational polarization within the testes' cells, for the control and under the effect of AM and ASE interactions within the testes' cells. As can be seen in the lower part of Fig. 4 it is greatly damped by AM interactions with cellular molecules to reach about 2 Hz. This means AM interactions complicate the environment within the testes' cells entangling too many molecules chemically and physically together. Leading to severe delays in charge movements and complicating molecules into larger polymeric ones. Which results in raising the relaxation peak strength near 700 M Ω in AM group

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instead of about 6 k Ω in the control. Both modalities of ASE use succeeded in retaining the relaxation peak to values around its control values with more success to the therapeutic ASE doses and the lower dose of protective ASE. Data mean that ASE components either were able and/ or enabled the biosystem to counteract most, if not all, of AM damaging interactions within the testes' cells.

3.2.2 Relaxation time of testes:

Fig. 5, shows the relaxation time of testes with temp. range (283-313K) of protected ASE groups (G3-5) in the upper part, and therapeutic ASE groups (G6-8) in the lower part compared to control (G1), and AM (G2) groups.

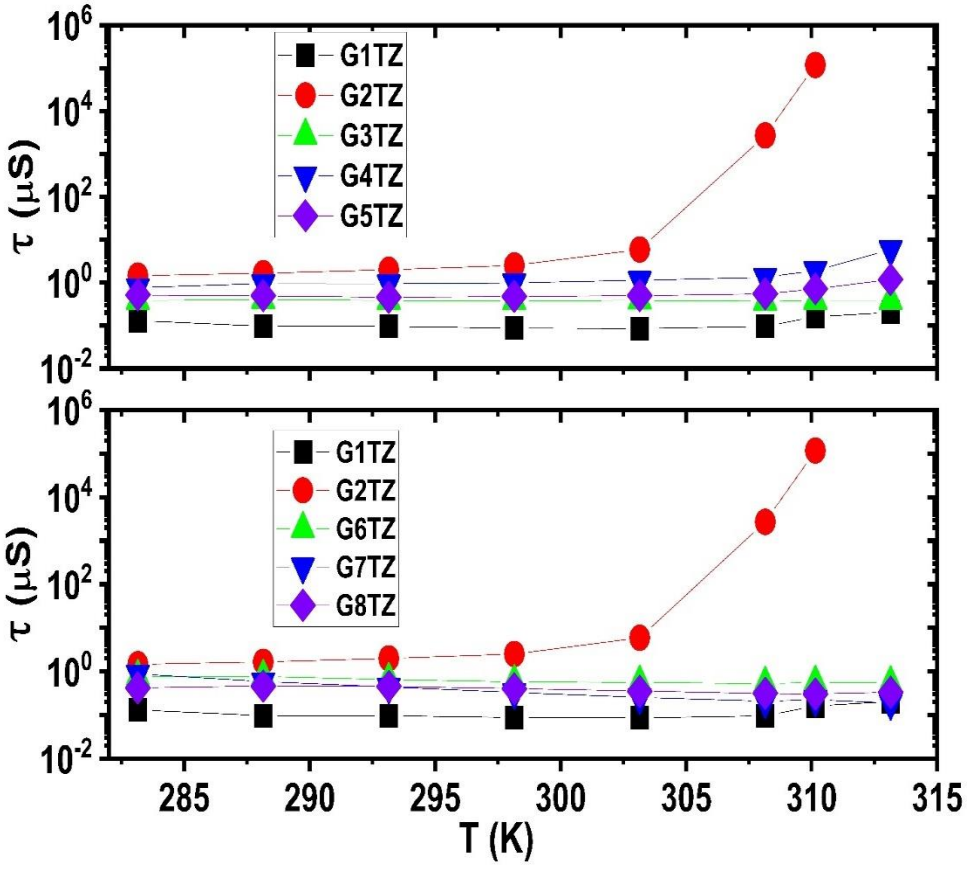

Fig. 5. Relaxation time of testes in temp. range (283-313K) of protected ASE groups (G3-5) (upper part), and therapeutic ASE groups(G6-8) (lower part) compared to control (G1), and AM (G2) groups.

From Fig. 5, the results showed that AM increased the relaxation time of the appointed process more than tenfold. The protective doses of ASE (G3-5) reduced the relaxation time values compared to the AM effect. The best results were of the lower and higher doses. ASE as a therapeutic agent, after ceasing AM, (G6-8), also decreased the relaxation times, where the intermediate and high doses competed about how close the relaxation time to control. At physiological temperatures (308-313K), the τ values of therapeutic groups were the nearest to control.

It's suggested that protein linkages and other interactions that AM does within cells, including lipid oxidation, protein aggregation, and vacuolar degeneration of spermatogenesis cells led to interruption and obstruction of many cellular processes; one of them is the relaxation time.

Interference of ASE as a protective and therapeutic agent helped the testes tissue to restore τ values near control. This indicated ASE which contains antioxidants such as polyphenols and flavonoids, can reduce the damage of spermatogenesis cells.

Moreover, as the results of ASE effects do not follow dose concentration, the ASE effect is more complicated and requires separate investigations to explore its potential. As seen from unpublished data, it has a special effect on the electrical properties of the tissues under study. More or less, both cases could oppose the AM interaction and recover the tissue near control.

3.2.3 Electrical conductivity of testes:

Fig. 6, displays the testes conductivity of direct current, σ_{dc}, of protected ASE groups (G3-5) (upper part), and therapeutic ASE groups (G6-8) (lower part), compared to control (G1), and AM (G2) groups with temp. range (283-313K).

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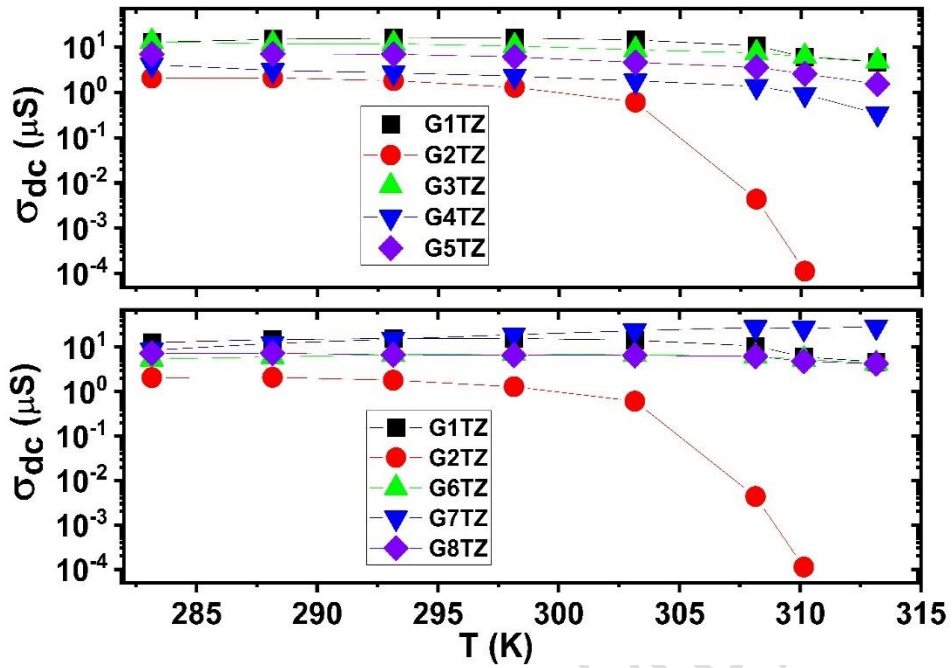

Fig. 6. Electrical conductivity, $\sigma_{dc,}$ of testes in temp. range (283-313K) of protected ASE groups (G3-5) (upper part), and therapeutic ASE groups (G6-8) (lower part) compared to control (G1), and AM (G2) groups.

In Fig. 6, the results indicate that AM interactions within testes' cells decreased DC conductivity, G2, while the ASE protective doses, G3-5, increased dc conductivity compared to AM group. The lowest ASE dose showed the best results followed by the highest and the intermediate doses, respectively. Also, the ASE therapeutic doses increased dc conductivity, G6-8, compared to G2. The highest and lowest doses had comparable results below the control level. The intermediate, on the other hand, had higher DC conductivity than control at physiological temperatures.

DC conductivity data of the testes indicated that the ASE has its own effect alongside its antagonist effect on AM. The lowest ASE dose could restore control DC conductivity, and the highest dose was near the control level, at physiological temperatures, for both protective and therapeutic ASE. This leads us to conclude that ASE effect is not linear. It needs another designed experiment to unleash and investigate its mode of action on different tissue types. The bleeding shown in histopathology in therapeutic cases indicated the AM damage and revealed the powerful effect of ASE as it prevented bleeding when administered with AM, protective use. While ASE restored the

values of DC conductivity at physiological temperatures, despite the presence of bleeding during the therapeutic use.

3.2.4 Activation energy of testes:

Fig. 7, pointed to the activation energy of testes, Y axis, extracted from relaxation time, τ, and DC conductivity, σ_{dc}, of protected ASE groups (G3-5), and therapeutic ASE groups(G6-8), compared to control (G1), and AM (G2) groups, X axis.

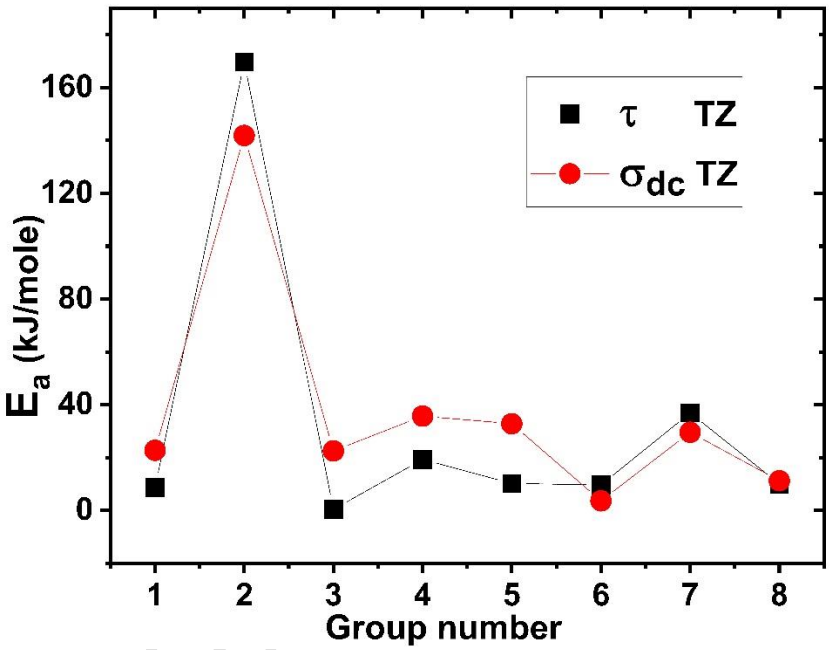

Fig. 7. Activation energy of testes deduced from τ and σ_{dc} of protected ASE groups (G3-5), and therapeutic ASE groups (G6-8), compared to control (G1), and AM (G2) groups.

From Fig. 7, AM hugely raised the activation energy for relaxation time and DC conductivity. Both protective and therapeutic ASE doses decreased the activation energy of both processes. The lower protective dose (G3τ) had the lowest activation energy, lower than the control, for the relaxation time. Alternatively, σ_{dc} for the lower therapeutic dose (G6) got the lowest activation energy, also lower than control, for the DC conductivity. Intermediate doses (G4, 7) for both the protective and therapeutic doses had the highest activation energy for both processes; still within the range of control's

activation energy. Therefore, ASE helped the biosystem to oppose AM action in a dose-independent fashion.

3.2.5 Enthalpy:

Fig 8, explores the testes enthalpy change of protected ASE groups (G3-5) (upper part), and therapeutic ASE groups (G6-8) (lower part) compared to control (G1), and AM (G2) groups with temp. range (283-313K).

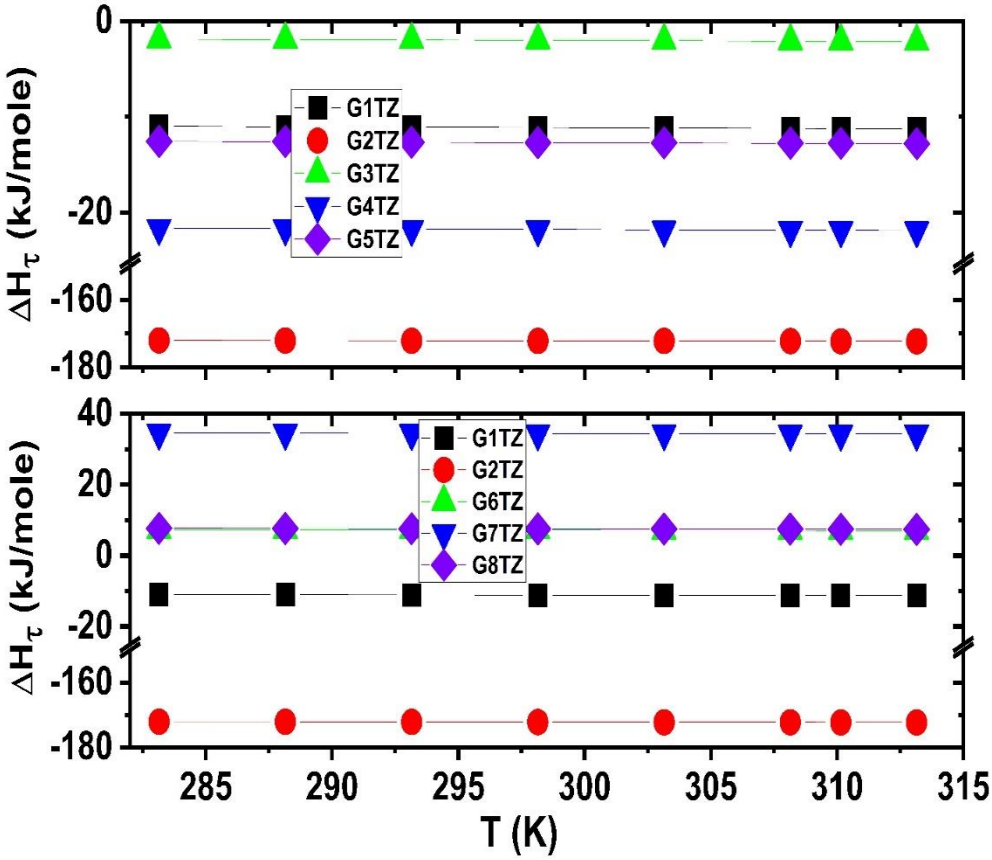

Fig. 8. Enthalpy of testes in temp. range (283-313K) of protected ASE groups (G3-5) (upper part), and therapeutic ASE groups(G6-8) (lower part) compared to control (G1), and AM (G2) groups.

The results of Fig. 8, pointed out the decrement of testes enthalpy change caused by AM interactions within testes' cells expressing the workload applied by AM and the performed work and heat evolved by testes' tissues to overcome these interactions and their impacts. Administering ASE with AM as protective doses, G3- 5, helped the biosystem to reverse or neutralize the AM effect on testes. The best results were of the high dose (G5), where it could restore the control level.

Therapeutic use of ASE, G6-8, indicated all doses of ASE could reverse the AM effect. ASE intermediate therapeutic dose, G7, had the highest enthalpy change. Also, both low and high therapeutic doses, G6 and G8 respectively, had the same effect of raising the enthalpy change to positive values above the control level, and they have the nearest values to control. Elevating the enthalpy change to positive values pointed to that all therapeutic doses enabled the testes tissues to gain heat from surrounding media. Therefore, these data show that ASE, in both cases, helped the testes tissues overcome the AM effect and only the highest dose of protective ASE could nearly restore the control values.

From all previous findings, it was observed that the histopathological analysis explored that ASE doses, generally, helped the testes tissue to be more intact, but the protective ASE was more helpful as it could cease the interstitial hemorrhage. Additionally, the dielectric and thermodynamic parameters showed that the results of protective and therapeutic ASE have similar behavior. Impedance, relaxation time, and DC conductivity displayed that therapeutic ASE was more helpful for restoring the testes tissue to normal case at physiological temperatures. Activation energy has no significant difference, while the enthalpy change showed that the highest dose of protective ASE could restore this parameter close to control values.

Finally, as the physical changes of biosystem appear faster than the biological ones, so, the results of physical parameters predict that the testes samples of histopathology can be more intact and have no hemorrhage upon the use of therapeutic ASE for a longer duration than 7 days after ceasing the AM administration.

Conclusion:

In this work, amoxicillin-induced testicular toxicity was noted in the testis histopathology, including severe depletion of spermatogenetic cells, vacuolar cellular degeneration, and hemorrhage in the interstitial tissues. Conversely, the testes' tissue samples were more intact in the protected and therapeutic ASE groups, and it was more apparent in the protected one. This means ASE could diminish AM influence and partially prevent tissue injuries.

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Also, the present work revealed the efficiency of dielectric and thermodynamic parameters in differentiation between the impacts of amoxicillin toxicity on the testes, and also the distinction among the protective and therapeutic groups of ASE in fighting the AM effects.

Some parameters such as relaxation time and conductivity showed the role of therapeutic ASE was more effective than the protective one. While other parameters such as activation energy displayed the same influence of both. The physical parameters predict that the testes tissue will improve more upon using therapeutic ASE for a longer duration than 7 days after ceasing the AM administration. Generally, Ashwagandha seeds proved their ability to protect the testicular tissue against cytotoxicity of amoxicillin whether as a protective or therapeutic agent. Therefore, it's suggested that ASE can be described along with antibiotics treatment to diminish the adverse effects of these drugs.

References

1. Hargreaves CA, Rogers S, Hills F, Rahman F, Howell RJ, Homa ST. Effects of co-trimoxazole, erythromycin, amoxycillin, tetracycline and chloroquine on sperm function in vitro. Hum Reprod. 1998 Jul;13(7):1878-86. doi: 10.1093/humrep/13.7.1878. PMID: 9740442.
2. Aral F, Karaçal F, Baba F. The effect of enrofloxacin on sperm quality in male mice. Res Vet Sci. 2008 Feb;84(1):95-9. doi: 10.1016/j.rvsc.2007.04.007. Epub 2007 Jun 11. PMID: 17561208.
3. Farombi EO, Ugwuezunmba MC, Ezenwadu TT, Oyeyemi MO, Ekor M. Tetracycline-induced reproductive toxicity in male rats: effects of vitamin C and N-acetylcysteine. Exp Toxicol Pathol. 2008 Jun;60(1):77-85. doi: 10.1016/j.etp.2008.02.002. Epub 2008 Apr 11. PMID: 18406588.
4. Antohi E, Gales C, Nechifor M. Pharmacological agents that affect sperm motility. Rev Med Chir Soc Med Nat Iasi. 2011 Oct-Dec;115(4):1183-8. PMID: 22276467.
5. Bouchicha A. El B., Kalem A., Mimoune N., Djouadi, S., Khelef D.and Kaidi R. Study of antibiotics and symbiotic effects on sperm quality using the CASA system, VETERINARSKA STANICA. 2022, 53 (4), https://doi.org/10.46419/vs.53.4.2
6. Liu Y, Liu Y, Kong Z., Cheng P., Zhu L., Wang H. Developmental toxicity window of fetal testicular injury in offspring mice induced by prenatal amoxicillin exposure at

Egypt J. Solids, Vol. (46) (2024)

different time, doses and courses, Toxicology Letters. 2023, 374: 85–95, https://doi.org/10.1016/j.toxlet.2022.12.006

7. Mahdi AA, Shukla KK, Ahmad MK, Rajender S, Shankhwar SN, Singh V, Dalela D. Withania somnifera Improves Semen Quality in Stress-Related Male Fertility. Evid Based Complement Alternat Med. 2009 Sep 29;2011:576962. doi: 10.1093/ecam/nep138. Epub ahead of print. PMID: 19789214; PMCID: PMC3136684.

8. Shukla KK, Mahdi AA, Mishra V, Rajender S, Sankhwar SN, Patel D, Das M. Withania somnifera improves semen quality by combating oxidative stress and cell death and improving essential metal concentrations. Reprod Biomed Online. 2011 May;22(5):421-7. doi: 10.1016/j.rbmo.2011.01.010. Epub 2011 Mar 8. PMID: 21388887.

9. Gupta A, Mahdi AA, Shukla KK, Ahmad MK, Bansal N, Sankhwar P, Sankhwar SN. Efficacy of Withania somnifera on seminal plasma metabolites of infertile males: a proton NMR study at 800 MHz. J Ethnopharmacol. 2013 Aug 26;149(1):208-14. doi: 10.1016/j.jep.2013.06.024. Epub 2013 Jun 21. PMID: 23796876.

10. Rahmati B, Ghosian Moghaddam MH, Khalili M, Enayati E, Maleki M, Rezaeei S. Effect of Withania somnifera (L.) Dunal on Sex Hormone and Gonadotropin Levels in Addicted Male Rats. Int J Fertil Steril. 2016 Jul-Sep;10(2):239-44. doi: 10.22074/ijfs.2016.4915. Epub 2016 Jun 1. PMID: 27441058; PMCID: PMC4948077.

11. Durg S, Shivaram SB, Bavage S. Withania somnifera (Indian ginseng) in male infertility: An evidence-based systematic review and meta-analysis. Phytomedicine. 2018 Nov 15; 50:247-256. doi: 10.1016/j.phymed.2017.11.011. Epub 2017 Nov 29. PMID: 30466985.

12. Paudel S., Aryal, B. and Ranabhat S. Amoxicillin induced behavioral neurotoxicity and histopathological changes in organs of Albino rats. Journal of College of Medical Sciences-Nepal. Jan-Mar 2020, 16(1): 12- 16.

13. Maheswari, R. and Manisha, P. Withania somnifera L root extract ameliorates toxin induced cytotoxicity. International Journal of Pharma Sciences and Research. May 2015, 6(5): 848- 855. http://www.ijpsr.info/docs/IJPSR15-06-05-020.pdf

14. Bancroft JD, Gamble M. *Theory and practice of histological techniques*. Vol. **172**. 5th ed. Edinburgh: Churchill Livingstone Pub; 2002 pp. 593–620

15. Idris, N. H., Majid, S. R., Khiar, A. S. A., Hassan, M. F. & Arof, A. K. Conductivity studies on chitosan/PEO blends with LiTFSI salt. Ionics 2005, 11(5–6), 375–377. DOI https://doi.org/10.1007/bf02430249.

16. Płowas, I., Swiergiel, J., & Jadzyn, J. Electrical conductivity in dimethyl sulfoxide **þ** potassium iodide solutions at different concentrations and temperatures. Journal of Chemical & Engineering Data 2014, 59, 2360–2366.

Egypt. J. Solids, Vol. (46), (2024)

17. Shamsudin, I. J., Ahmad, A., Hassan, N. H., & Kaddami, H. Bifunctional ionic liquid in conductive biopolymer based on chitosan for electrochemical devices application. Solid State Ionics. October 2015, 278, 11–19

18. Elwan AM, Salama AA, Sayed AM, Ghoneim AM, Assaied AA, Ibrahim FA, Shousha HA, Elnasharty MMM. Response of rats to dose rates of ionizing radiation evaluated by dielectric properties of bone marrow. Prog Biophys Mol Biol. 2018 Dec; 140:124-132. doi: 10.1016/j.pbiomolbio.2018.05.007. Epub 2018 May 10. PMID: 29776659.

19. Elwan AM, Salama AA, Sayed AM, Ghoneim AM, Elsaied AA, Ibrahim FA, Elnasharty MMM. Biophysical and biochemical roles of Moringa oleifera leaves as radioprotector. Prog Biophys Mol Biol. 2018 Dec; 140:142-149. doi: 10.1016/j.pbiomolbio.2018.06.003. Epub 2018 Jun 6. PMID: 29885346.

20. Elnasharty, Mohamed M. M. and Azhar M. Elwan. "Evaluation of Protective Effects of Moringa Leaves Extract on BM Cells Injury Caused by Radiation Using Thermodynamic State Functions Derived Dielectric Parameters." BioTechnology: An Indian Journal 2018, 14 (5): 1-10. https://www.tsijournals.com/articles/evaluation-of-protective-effects-of-moringa-leaves-extract-on-bm-cells-injury-caused-by-radiation-using-thermodynamic-st.pdf

21. Elnasharty, M. M. M., & Elwan, A. M. Estimation of radiation dose rates' changes on Hb using dielectric parameters. Arab Journal of Basic and Applied Sciences 2019, 26(1), 236–241. https://doi.org/10.1080/25765299.2019.1607996

22. Elnasharty, M., Elwan, A. "Influence of Moringa Leaves Extract on the Response of Hb Molecule to Dose Rates' Changes: II. Relaxation Time and Its Thermodynamic Driven State Functions". World Academy of Science, Engineering and Technology, Open Science Index 149, International Journal of Medical and Health Sciences 2019, 13(5), 261 - 265.

23. Elnasharty M M, Elwan A. M. Dielectrically driven thermodynamic state functions discriminating bone marrow response for two radiation dose rates, Measurement 2023, 217, 113023. https://doi.org/10.1016/j.measurement.2023.113023.

24. Elnasharty MMM, Elwan AM. Dielectric investigation of irradiated RBCs and study the role of Moringa leaves extract against radiation damage. Appl Radiat Isot. 2023 Jun; 196:110776. doi: 10.1016/j.apradiso.2023.110776. Epub 2023 Mar 13. PMID: 36947911.

25. Elnasharty MMM, Elwan AM, Elhadidy ME, Mohamed MA, Abd El-Rahim AH, Hafiz NA, Abd-El-Moneim OM, Abd El-Aziz KB, Abdalla AM, Farag IM. Various investigations of ameliorative role of Ashwagandha seeds (*Withania somnifera*) against

Egypt J. Solids, Vol. (46) (2024)

amoxicillin toxicity. Toxicol Res (Camb). 2024 Jun 12;13(3): tfae091. doi: 10.1093/toxres/tfae091. PMID: 38873278; PMCID: PMC11167568.

26. Al-Jowari, S. A. Comparative investigation of amoxicillin and cephalexin administration on some biochemical parameters in male rabbits. *IJSR*. 2018, *7*, 1481-1484. https://api.semanticscholar.org/CorpusID:53559291

27. Žura Žaja, I., M. Samardžija, S. Vince, I. Majićbalić, D. Đuričić and S. Milinković-tur. Antioxidant system parameters in boar spermatozoa of different morphology and motility. Vet. Arhiv. 2016, 86: 655-666. https://hrcak.srce.hr/167551

28. Žura Žaja, I., M. Samardžija, S. Vince, M. Vilić, I. Majić-balić, D. Đuričić and S. Milinković-tur. Differences in seminal plasma and spermatozoa antioxidative systems and seminal plasma lipid and protein levels among boar breeds and hybrid genetic traits. Anim. Reprod. Sci. 2016, 170, 75-82.10.1016/j.anireprosci.2016.04.006. https://doi.org/10.1016/j.anireprosci.2016.04.006.

29. Žura Žaja, I., A. Sluganović, M. Samardžija, S. Milinković-tur, T. Dobranić, S. Strelec, D. Đuričić, H. Valpotić and S. Vince. System and mechanisms of antioxidant protection. Vet. Stn. 2019, 50, 43-54. (In Croatian).

30. Žura Žaja, I., I. Švub, S. Milinković-tur, S. Vince, H. Valpotić, D. Đuričić, S. Čipčić and M. Samardžija. Biochemical parameters of seminal plasma in domestic animals and humans. Vet. stn. 2019, 50, 223-237. (In Croatian). https://hrcak.srce.hr/223829

31. Torres-Arce E, Vizmanos B, Babio N, Márquez-Sandoval F, Salas-Huetos A. Dietary Antioxidants in the Treatment of Male Infertility: Counteracting Oxidative Stress. Biology (Basel). 2021 Mar 20;10(3):241. doi: 10.3390/biology10030241. PMID: 33804600; PMCID: PMC8003818.

32. Ahmad MK, Mahdi AA, Shukla KK, Islam N, Rajender S, Madhukar D, Shankhwar SN, Ahmad S. Withania somnifera improves semen quality by regulating reproductive hormone levels and oxidative stress in seminal plasma of infertile males. Fertil Steril. 2010 Aug;94(3):989-96. doi: 10.1016/j.fertnstert.2009.04.046. Epub 2009 Jun 6. PMID: 19501822.

33. Kumar A, Kumar R, Rahman MS, Iqubal MA, Anand G, Niraj PK, Ali M. Phytoremedial effect of Withania somnifera against arsenic-induced testicular toxicity in Charles Foster rats. Avicenna J Phytomed. 2015 Jul-Aug;5(4):355-64. PMID: 26445714; PMCID: PMC4587609.

34. Darbandi M, Darbandi S, Agarwal A, Sengupta P, Durairajanayagam D, Henkel R, Sadeghi MR. Reactive oxygen species and male reproductive hormones. Reprod Biol

Egypt. J. Solids, Vol. (46) (2024)

Endocrinol. 2018 Sep 11;16(1):87. doi: 10.1186/s12958-018-0406-2. PMID: 30205828; PMCID: PMC6134507.

35. Karaman M, Budak H, Çiftci M. Amoxicillin and gentamicin antibiotics treatment adversely influence the fertility and morphology through decreasing the *Dazl* gene expression level and increasing the oxidative stress. Arch Physiol Biochem. 2019 Dec;125(5):447-455. doi: 10.1080/13813455.2018.1482354. Epub 2018 Jun 20. PMID: 29925282.

36. Kumar Y. S., Kumari N. S., Sharmila K. P., and Bhargavan D. Cyclophosphamide induced DNA and testicular damage: protective role of *withania somnifera*, IJPSR. 2023; 14(4): 1762-1768. DOI: 10.13040/IJPSR.0975-8232.14(4).1762-68

37. Aboelhassan, Dalia M., Noha E. Ibrahim, Mohamed M. M. Elnasharty, Azhar M. Elwan, Mohamed E. Elhadidy, Mona A. Mohamed, Hasnaa A. Radwan, Inas S. Ghaly and Ibrahim M. Farag. Biological and physical studies on the protective and therapeutic roles of ashwagandha seed extract against the potential toxic effect of amoxicillin in rats. Egyptian Pharmaceutical Journal 2024, 23(2): 251-263. https://api.semanticscholar.org/CorpusID:269390595

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