Slov Vet Res 2023; 60 (Suppl 25): 433–38
DOI 10.26873/SVR-1673-2023

Original Research Article
Veterinary Medicine and The One Health Concept

AMELIORATIVE EFFECTS OF VITAMIN E AND SELENIUM ON BLEOMYCIN-INDUCED MALE INFERTILITY

Hosny Abd El-Fadil Ibrahim[1], Shimaa I. Shalaby[2], Ahmed Abdelfattah-Hassan[3], Rasha Mahmoud M. A. Hebishy[4]*, Eman Mohamed Abdel Mohsen Abdel Ghani[1]

[1]Pharmacology Department, [2]Physiology Department, [3]Department of Anatomy and Embryology, [4]Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt

*Corresponding author, E-mail: rashamahmoud073@gmail.com

Abstract: Bleomycin (BL) is a well-known anticarcinogenic chemotherapy that is used for the remedy of numerous varieties of most cancers. However, the use of BL became connected to the prevalence of several unfavorable results together with skin and lung fibrosis and testicular cancer. Dietary micronutrients such as vitamin E, and selenium (Se) are gambling critical roles in maintaining the health of human and animals and preserving the cell redox status homeostasis through their antioxidant roles. The objectives of the current investigation had been to investigate BL-induced male infertility in Albino rats. The protecting effects of either vitamin E, or Se towards such detrimental outcomes had been additionally examined. Moreover, the potential mechanisms behind such adverse effects had been evaluated. The obtained results indicated significant reduction in the testosterone, FSH, and LH levels in the BL-treated group. Likely, sperm characteristics as sperm count and motility had been significantly reduced in BL-treated group. Such changes have been associated with a significant reduction within the antioxidant enzymes including CAT, GPx, SOD, and MDA. Interestingly, co-exposure of experimental rats to either BL with vitamin E, or Se had notably parameters relatively close to the normal levels suggesting the ameliorative effects of vitamin E and Se towards BL-induced male infertility.

Key words: prevalence; Bleomycin; selenium; vitamin E; vale fertility

Introduction

Human cancer is frequently treated with bleomycin (BL), a glycopeptide antibiotic produced from the bacteria *Streptomyces verticillus*. In the past 20 years, early identification and improved cancer treatment protocols have significantly boosted the percentage of men who survive cancer at a young age; now, more than 75% of young cancer patients are long-term survivors. However, after receiving chemotherapy and radiation treatment, fertility is commonly compromised (1). In this context, BL is linked to several incidences of male infertility in both humans and animals (2-5).

Received: 28 December 2022
Accepted for publication: 4 January 2023

By reacting with free radicals to generate a tocopheryl radical, which is then reduced by a hydrogen donor and returns to its reduced state, vitamin E functions as an antioxidant by acting as a peroxyl radical scavenger and limiting free radical proliferation in tissues. Due to its solubility, it is absorbed into cell membranes, preventing oxidative damage (6). Due to the high amount of polyunsaturated fatty acids (PUFA) in their membranes, spermatozoa and germ cells are particularly susceptible to oxidation. Vitamin E is a strong lipophilic chain-breaking antioxidant that shields tissue PUFA from peroxidation (7, 8).

For good spermatogenesis, spermatozoa motility, -and testicular development, selenium (Se) is an essential mineral. In both humans and animals, selenium predominantly guards cellular

membranes and organelles against oxidative damage by functioning as an antioxidant through the Se-dependent enzyme glutathione peroxidase (9). A slight deviation, either a lack or an excess of Se in the testis, causes the male reproductive tissue, which depends on an appropriate amount of Se in the testis, to develop improperly. When spermatozoa mature, selenium helps selenoproteins like GPx1, GPx3, mGPx4, cGPx4, and GPx5 protect them from oxidative damage (10).

Infertility affects 15% of couples and in 30% of these cases, the male partner's abnormalities—known as male infertility—are to blame. The oxidative damage caused by an increase in reactive oxygen species is now being linked to numerous cases of male infertility that were previously assumed to be idiopathic. This type of infertility is indicated to be treated with oral Se, and vitamin E supplementation (11).

In sight of the previous facts, this study aimed to investigate the adverse effects of BL on the male infertility using Albino rats as a model. Besides, the ameliorative effects of Vitamin E and Se against BL-induced male infertility in Albino rats had been evaluated. Moreover, the possible mechanisms behind such effects were also examined.

Material and methods

All experiments using animals were done according to the animal use guidelines of Zagazig University, Egypt. This study received an ethical approval number ZU-IACUC/2/F/115/2021.

Animals:

Forty albino male healthy rats, each weighing 180±10 g, were utilized in this investigation. Animals were acquired from the Animal House at Faculty of Veterinary Medicine, Zagazig University, Egypt. Before beginning the research work, the animals were kept under observation and given a week to get used to the lab setting. They were housed in metal cages under hygienic conditions, fed laboratory animal ration during the trial, and given free access to water.

Experimental design, Animals, and treatment:

Animals were grouped into four groups (n = 10 animals/group). Animals in group 1 received distilled water via intraperitoneal injection at a dose of 0.2 mL/animal and served as a control group. Animals in group 2, animals received bleomycin at 1.5 IU/kg BW via intraperitoneal injection twice weekly for three successive weeks. Animals in group 3 received Vitamin E (100 mg/kg BW) orally two hours prior to the administration of bleomycin at 1.5 IU/kg BW via intraperitoneal injection twice weekly for three successive weeks. Animals in group 4 received selenium (0.2 mg/kg BW) orally two hours prior to the administration of bleomycin at 1.5 IU/kg BW via intraperitoneal injection twice weekly for three successive weeks. Animals had given a free excess to feed and water. The environmental conditions of the animal room were at 23–25 °C, 12 hr light/dark cycle, and 50 ± 10% relative humidity. Pentobarbital sodium (40 mg/kg, 0.036 g/mL) was used to anaesthetize each animal before it was weighed and sacrificed. The serum was separated by centrifuging blood samples for 10 min. at 1500 rpm and 4 °C. Blood samples were taken from the retro-orbital sinus. After that, serum was kept at -80 °C for upcoming biochemical tests. The cauda epididymis of the left testis was dissected and transferred to sterilized Petri dish containing 2 ml warm normal saline at 37 °C, then macerated by sterilized scissor to obtain a suspension of the epididymal content (12).

Estimation of reproductive hormones:

Serum testosterone level was estimated by using rat ELISA kit (Cusabio, China), while LH, and gametogenic hormone (FSH) levels were determined by using rat ELISA kit purchased from MY–BIO Source (San Diego, CA) according to the manufacturer's protocol (13).

Analysis of sperm characteristics:

Sperm characteristics including count, motility and sperm abnormalities were analyzed according to the established methods (14, 15).

Measurement of oxidant/antioxidant enzyme assays in the testicular tissue:

Measurement of the antioxidant enzymes including catalase (CAT), glutathione peroxidase (GPX), malondialdehyde (MDA), and superoxide

dismutase level in the testicular tissue was done using specific Biodiagnostic kits according to the manufacturer's guides (16).

Statistical analysis:

The results were shown as means ± standard error (SE). The data were subjected to a one-way analysis of variance (ANOVA) at a 95% level of confidence using the statistical package for social sciences (SPSS-16: Chicago, IL, USA) software followed by Tukey's Kramer HD test, where $P < 0.05$ was considered as significant.

Results

The recorded results in Table 1 showed that BL significantly reduced reproductive hormones including testosterone, FSH, and LH after both 30 and 60 days of treatment. While co-exposure of rats to either vitamin E and BL, or Se and BL could significantly restore the reproductive hormones to their normal levels or even increase their secretion.

The obtained results in Table 2 demonstrated that BL caused significant reduction in the sperm counts and motilities with a significant increase in the total abnormalities. Interestingly, co-exposure of rats to either vitamin E and BL, or Se and BL could significantly increase the sperm count and motility and reduce the total abnormalities.

The recorded results in Table 3 showed that BL caused significant reduction in the testicular antioxidant enzymes including CAT, GPx, and SOD with significant increase in MDA production. Unlikely, co-exposure of rats to either vitamin E and BL, or Se and BL could significantly increase the CAT, GPx, and SOD levels with significant reduction in the MDA levels.

Table 1: Effect of Vitamin E (100 mg/kg BW) and Selenium (0.2 mg/kg BW) orally twice weekly for three successive weeks on serum testosterone, FSH and LH level in adult male rats treated with bleomycin (1.5 IU/kg BW I/P twice weekly for three successive weeks) after 30 and 60 days

Groups	Testosterone (ng/mL)		FSH (mLu/mL)		LH (mLu/mL)	
	After 30 days	After 60 days	After 30 days	After 60 days	After 30 days	After 60 days
Control	4.65 ± 0.29^{b}	5.43 ± 0.57^{b}	2.49 ± 0.29^{b}	2.63 ± 0.32^{a}	11.27 ± 0.37^{b}	11.93 ± 0.64^{ab}
BL	0.53 ± 0.02^{c}	0.45 ± 0.05^{c}	0.76 ± 0.03^{c}	0.63 ± 0.06^{b}	6.53 ± 0.85^{c}	8.50 ± 1.51^{b}
Vit. E + BL	8.74 ± 0.32^{a}	8.55 ± 0.33^{a}	4.33 ± 0.49^{a}	4.10 ± 0.59^{a}	15.17 ± 0.66^{a}	15.10 ± 0.67^{a}
Se + BL	5.63 ± 0.37^{b}	6.21 ± 0.43^{b}	2.60±0.35^{b}	3.13 ± 0.19^{a}	11.40 ± 0.36^{b}	13.20 ± 0.44^{a}

Values within the same column carrying different superscript letter are significantly different at P< 0.05

Table 2: Effect of Vitamin E (100 mg/kg b. wt) and Selenium (0.2 mg/kg b. wt) orally twice weekly for three successive weeks on sperm characters (Sperm count, sperm motility and sperm abnormalities) in adult male rats treated with bleomycin (1.5 IU/kg b. wt I/P twice weekly for three successive weeks) after 30 and 60 days

Groups	Sperm count × 10^{6}/mL		Motility (%)		Total abnormalities (%)	
	After 30 days	After 60 days	After 30 days	After 60 days	After 30 days	After 60 days
Control	61.67 ± 4.41^{b}	60.00 ± 5.77^{b}	46.00 ± 8.89^{b}	47.50 ± 3.04^{b}	9.53 ± 1.12^{b}	13.83 ± 1.17^{b}
BL	35.00 ± 2.52^{c}	29.00 ± 2.08^{c}	24.10 ± 3.16^{c}	17.63 ± 2.86^{c}	12.90 ± 0.59^{a}	19.33 ± 0.67^{a}
Vit. E + BL	76.67 ± 3.33^{a}	86.67 ± 3.33^{a}	71.67 ± 4.33^{a}	81.73 ± 2.91^{a}	7.90 ± 0.10^{b}	10.07 ± 0.64^{b}
Se + BL	75.67 ± 2.96^{a}	78.33 ± 1.67^{a}	67.47 ± 4.17^{a}	71.83 ± 2.46^{ab}	8.57 ± 0.52^{b}	12.67 ± 1.30^{b}

Values within the same column carrying different superscript letter are significantly different at P< 0.05

Table 3: Effect of Vitamin E (100 mg/kg b. wt) and Selenium (0.2 mg/kg b. wt) orally twice weekly for three successive weeks on antioxidant/oxidant status (CAT, GPX, SOD and MDA) in adult male rats treated with bleomy-cin (1.5 IU/kg b. wt I/P twice weekly for three successive weeks) after 30 and 60 days

	CAT (u/g)		GPx (mu/mL)		SOD (u/mg)		MDA (nmol/g)	
	After 30 days	After 60 days	After 30 days	After 60 days	After 30 days	After 60 days	After 30 days	After 60 days
Control	1.43 ± 0.07[b]	1.63 ± 0.09[a]	6.10 ± 0.09[a]	6.10 ± 0.09[a]	77.33 ± 2.73[a]	78.00 ± 3.21[a]	0.40 ± 0.04[c]	0.43 ± 0.01[b]
BL	0.71 ± 0.15[c]	0.67 ± 0.16[b]	3.43 ± 0.23	2.90 ± 0.38[b]	41.67 ± 0.88	40.67 ± 0.88[d]	4.64 ± 0.52[a]	5.66 ± 1.1[a]
Vit. E + BL	2.13 ± 0.06[a]	2.13 ± 0.07[a]	4.47 ± 0.27	4.97 ± 0.35[a]	52.67 ± 1.45	56.00 ± 1.15[c]	2.07 ± 0.09[b]	2.07 ± 0.23[b]
Se + BL	1.67 ± 0.09[b]	1.83 ± 0.17[a]	4.80 ± 0.17	4.94 ± 0.20[a]	59.33 ± 0.88	62.33 ± 2.33[b]	2.00 ± 0.25[b]	1.87 ± 0.23[b]

Values within the same column carrying different superscript letter are significantly different at P< 0.05

Discussion

Bleomycin is a glycopeptide antibiotic that is frequently used to treat human cancer. However, there are numerous instances of both human and animal male infertility that have been connected to BL. In the present study, BL caused significant reduction in the reproductive hormones such as testosterone, FSH, and LH. Besides, BL caused significant decrease in the sperm count and motility with an increase in the total sperm abnormalities. Such reproductive changes were associated with significant reduction in the testicular antioxidant enzymes with an increase in the oxidative damage as declared by the high MDA levels in the treatment groups compared with the control. Several cancer types have been treated with bleomycin, either alone or in conjunction with other chemotherapy drugs including etoposide and cis-platinum. In this regard, adult male and female Sprague-Dawley rats were treated with the therapeutically adequate dose levels of bleomycin, etoposide, and cis-platinum (BEP) in three cycles of 21 days each. Serum hormone levels, testicular histopathology, PCNA and transferrin expression, sperm characteristics, fertility, and testicular histopathology were all evaluated after the course of treatment. BEP caused sperm motility to decrease, sperm motility to decrease, abnormalities to rise, and tubular atrophy. elevated levels of inhibin B, but transferrin, FSH, and testosterone were lowered (17). In another study, the effects of BL on sperm parameters and the production of MDA in rats were evaluated. BL markedly increased the quantity of immature sperm, sperm with damaged DNA, and MDA concentration in comparison to the control group. On the other hand, BL markedly reduced the quantity, viability, and motility of sperm (2). In a different study, Balb/c mice in the BL-treated group received 10 mg/kg of BL intraperitoneally every day for 35 days. The results showed that BL significantly increased intermediate and immature non-progressive movement and immotile sperm while significantly decreasing sperm count, viability, morphology, maturation, and progressive movement, testosterone, the ratio of testis weight to body weight, the number of spermatogonia, spermatocytes, and Sertoli cells per tubule (18).

By interacting with free radicals to generate a tocopheryl radical, which is then reduced by a hydrogen donor and returns to its reduced state, vitamin E functions as an antioxidant and scavenger of peroxyl radicals, inhibiting their growth in tissues. Selenium is most likely an important substance that actively participates in a range of metabolic processes and has a number of significant physiological functions. Participation in different enzyme systems and antioxidant cellular processes are a couple of these roles. Vitamin E and selenium are very necessary micronutrients for the normal functions of the reproductive system. In co-exposure groups with either vitamin E and BL or Se and BL, significant improvements were achieved in the reproductive functions compared with BL-treated group. Interestingly the groups that received vitamin E and Se had higher levels of the reproductive hormones, sperm counts and motility. Such ameliorative effects were associated with a significant increase in the antioxidant enzymes in the testes. Similarly, the zona binding test shows that vitamin E taken orally greatly enhances the *in vitro* performance of human spermatozoa (19). Likely, in a study by Keskes-Ammar et al. (20) Vitamin E and selenium

supplements resulted in a considerable drop in MDA concentrations and an improvement in sperm motility. The findings support the use of vitamin E and selenium in the treatment of male infertility because they have protective and advantageous effects on the quality of semen. Furthermore, Se caused a significant improvement in the semen quality and secretion of testosterone in a human trial (21).

Conclusion

The current investigation demonstrated occurrence of BL-induced toxic effects on male reproductive organs leading to occurrence of male infertility as demonstrated by the significant reductions in the male hormones, and deviations in the sperm characteristics, such changes were associated with the induction of oxidative stress in the testicular tissue. Interestingly, vitamin E and selenium could ameliorate such adverse effects, possibly via upregulation of the testicular antioxidant enzymes.

Acknowledgments

All thanks to stuff members at Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Egypt for their technical support during practical part of this study.

Authors declare that they have no conflict of interest.

References

1. Dohle GR. Male infertility in cancer patients: review of the literature. Inter J Urol 2010; 17(4): 327–31.

2. Amirshahi T, Najafi G, Nejati V. Protective effect of royal jelly on fertility and biochemical parameters in bleomycin-induced male rats. Iran J Reprod Med 2014; 12(3): 209.

3. Khadivi F, Razavi S, Hashemi F. Protective effects of zinc on rat sperm chromatin integrity involvement: DNA methylation, DNA fragmentation, ubiquitination and protamination after bleomycin etoposide and cis-platin treatment. Theriogenol 2020; 142: 177–83.

4. Liu M, Hales BF, Robaire B. Effects of four chemotherapeutic agents, bleomycin, etoposide, cisplatin, and cyclophosphamide, on DNA damage and telomeres in a mouse spermatogonial cell line. Biol Reprod 2014; 90(4): 72–1.

5. Maselli J, Hales BF, Robaire B. The effects of chemotherapy with bleomycin, etoposide, and cis-platinum (BEP) on rat sperm chromatin remodeling, fecundity and testicular gene expression in the progeny. Biol Reprod 2013; 89(4): 85–1.

6. Gokce A, Oktar S, Koc A, et al. Protective effects of thymoquinone against methotrexate–induced testicular injury. Human Exper Toxicol 2011: 30: 897–903.

7. Bolle P, Evandri MG, Saso L. The controversial efficacy of vitamin E for human male infertility. Contraception 2002: 65(4): 313-–5.

8. Hashemi F, Razavi S, Khadivi F. The protective effects of omega3 on ubiquitination and protamination of rat sperm after bleomycin, etoposide, and cisplatin treatment. Nutr Cancer 2018; 70(8): 1308–14.

9. Moslemi MK, Tavanbakhsh S. Selenium–vitamin E supplementation in infertile men: effects on semen parameters and pregnancy rate. Inter J Gen Med 2011; 4: 99.

10. Ahsan U, Kamran Z, Raza I, et al. Role of selenium in male reproduction—A review. Anim Reprod Sci 2014; 146(1-2): 55–62.

11. Wen JC. The role of vitamin E in the treatment of male infertility. Nutr Bytes 2006; 11: 1.

12. Igweze ZN, Orisakwe OE, Obianime AW. Reproductive parameter in 90 day toxicity study of smart Herbal purifierR-a poly Herbal supplement in male rats. J Appl Pharm Cetri Sci 2014; 4(10): 069–74.

13. Burtis CA, Ashwood FR. Textbook of clinical chemistry, 2nd ED, WB Saunders Co., Philadephia, 1994; p1851.

14. Slott V, Suarez J, Perreault S. Rat sperm motility analysis: methodologic considerations. Reprod Toxicol 1991; 5: 449–58.

15. Filler R. Methods for evaluation of rat epidydimal sperm morphology. In: Methods in toxicology, vol.3 (part A). Male reproductive toxicology. Chapin RE, Heindel JJ (eds). Academic Press Limited, London, UK, 1993; 334–43.

16. Andersen RA, Ingebrigtsen K, Nafstad I, et al. Biochemical characteristics of rat superoxide dismutase and the effect caused by paraquat injection on the enzyme activity in various tissues. Gen Pharmacol 1984; 15(3): 205–10.

17. Kilarkaje N, Mousa AM, Al-Bader MM, et al. Antioxidants enhance the recovery of three cycles of bleomycin, etoposide, and cisplatin–induced

testicular dysfunction, pituitary-testicular axis, and fertility in rats. Fertil Steril 2013; 100(4): 1151–9.

18. Yaghutian Nezhad L, Mohseni Kouchesfahani H, Alaee S, et al. Thymoquinone ameliorates bleomycin-induced reproductive toxicity in male Balb/c mice. Human Exper Toxicol 2021; 40: S611–21.

19. Kessopoulou E, Powers HJ, Sharma KK, et al. A double-blind randomized placebo cross-over controlled trial using the antioxidant vitamin E to treat reactive oxygen species associated male infertility. Fertil Steril 1995; 64(4): 825–31.

20. Keskes-Ammar L, Feki-Chakroun N, Rebai T, et al. Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. Arch Androl 2003; 49(2): 83–94.

21. Safarinejad MR, Safarinejad S. Efficacy of selenium and/or N-acetyl-cysteine for improving semen parameters in infertile men: a double-blind, placebo controlled, randomized study. J Urol 2009; 181(2): 741–51.