Preserving the Normal Healthy Testis: Role of Beta-carotene

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Received	Accepted
1-10-2022	1-11-2022

ABSTRACT

Background: Many drugs can have significant deleterious side effects on the different organs of the body which can change normal physiology to different pathologies. Recent research have been focusing on valuable methods to preserve the normal healthy testis from the medications' adverse effects. The study aims to investigate the role of beta-carotene in the protection against potential amikacin's testicular toxicity.
Materials and methods: Male rats were involved in the study. Rats have divided into four groups: the first group was control, the second group has been given amikacin as an inducer of testicular toxicity; the third group has been given both amikacin and beta-carotene, and the fourth group has been given beta-carotene as prophylaxis before induction of the toxicity. The histological architecture of the testis was investigated for all the rats.
Results: Amikacin caused a significant deleterious effect which included degeneration and necrosis of cells of seminiferous tubules as well as atrophy and congestion of blood vessels. Co-administration of both beta-carotene and amikacin resulted in a partial improvement in the testicular tissue while using beta-carotene as prophylaxis succeeded in protecting and preserving the normal histological features of the testis completely.
Conclusion: The study concluded that beta-carotene can preserve the normal physiological testis and protect it against the amikacin's deleterious effect on the testis.

Keywords: Beta-carotene, Testis's preservation, seminiferous tubules.

الحفاظ على خصية طبيعية صحية: دور البيتاكاروتين

الخلاصة:
المقدمة: العديد من الادوية تمتلك القدرة على إحداث ثاثيرات ضارة في مختلف أعضاء الجسم والتي قد تؤدي الى تغيير من الفسلجة الطبيعية الى امراض مختلفة. تركز البحوث الحديثة على الطرق القيّمة للمحافظة على خصية طبيعية صحية من التأثيرات الجانبية للأدوية. الهدف من الدراسة هو فحص دور البيتاكاروتين في حماية انسجة الخصية من التأثيرات الضارة لدواء الاميكاسين.
طرق العمل: تم استخدام جرذان ذكور في الدراسة. تم تقسيم الجرذان الى 4 مجاميع: المجموعة الأولى كانت فقط للسيطرة. المجموعة الثانية أعطيت دواء الاميكاسين كمحفز للسمّية في الخصية. المجموعة الثالثة أعطيت كلا من دواء الاميكاسين والبيتاكاروتين. المجموعة الرابعة أعطيت جرعة وقائية من البيتاكاروتين قبل إعطاء دواء الاميكاسين والبيتاكاروتين. تم فحص الشكل النسيجي لجميع الجرذان.
النتائج: تسبب آعطاء دواء الاميكاسين بتأثيرات ضارة مهمة والتي تمثلت بتآكل وموت خلايا النبيبات المنوية بالإضافة الى ضمور واحتقان في الاوعية الدموية في الخصية. إعطاء البيتاكاروتين مع الميكاسين أدى الى تحسن جزئي في انسجة الخصية في حين نجح إعطاء جرعة وقائية إضافية من البيتاكاروتين قبل إعطاء كلا من الاميكاسين والبيتاكاروتين في الحماية والمحافظة على الصفات النسيجية للخصية وبصورة كلّيّة.
الاستنتاج: استنتجت الدراسة أنه بإمكان البيتاكاروتين الحفاظ على خصية طبيعية وحمايتها من الاثار الضارة لدواء الاميكاسين.

الكلمات المفتاحية: البيتاكاروتين، حفظ الخصية، الانابيب المنوية.

INTRODUCTION

Testes are the physiological male gonads that are responsible for spermatogenesis, the process of sperm production, and the production of sex hormones to maintain the normal physiological fertility status [1,2]. The germ cells are produced and matured in the lining of the seminiferous tubules in the testis. The physiological spermatogenesis requires two important functional cells Sertoli cells and Leydig cells. Leydig cells are present in the interstitium between seminiferous tubules and are responsible for the production of the principal male sex hormone testosterone [3]. However, Sertoli cells form a blood-testis barrier, keep germinal cells contained in the seminiferous tubules and promote spermatogenesis [4]. However, the physiology of testes and their secretions are changed due to several pathological conditions [5,6]. In addition, the testes can be adversely affected by many medications and toxins [7].

Aminoglycosides are antibiotics having potent antibacterial activity and are widely used in the treatment of many resistant infectious diseases [8]. They act against several gram-negative and gram-positive bacteria via the inhibition of bacterial protein synthesis [9]. The first member of this group, streptomycin, was discovered in 1944 when it was isolated from *Streptomyces griseus*. Then several members are identified including neomycin, kanamycin, gentamicin, netilmicin, tobramycin, and amikacin [10]. Amikacin has excellent antimicrobial activity against gram-negative bacilli from the Enterobacteriaceae family as well as *Acinetobacter baumanii* and *Pseudomonas aeruginosa* [11]. However, amikacin also has many harmful effects on different organs in the body including nephrotoxicity, ototoxicity, and, less commonly, neurotoxicity, drug fever, and rash [12]. Additionally, other physiological sites in the body can be affected by the deleterious effect of amikacin [13–15].

Many substances are discovered or produced to attenuate the adverse effects of the drugs and to preserve the normal physiological function of different affected organs[16–18]. One of these important substances is carotenoids which are found in food, especially yellow, green, and orange leafy fruits and vegetables (e.g., carrots, spinach), and also can be endogenously present in the human body [19,20]. Beta-carotene is an important carotenoid that acts as an antioxidant and has a beneficial effect in alleviating eye diseases [21]. In addition, recent research found that beta-carotene could have a role in enhancing the beneficial effect of the medication and protecting against the adverse effect of many drugs [22,23].

The study aims to investigate the potential beneficial effect of beta-carotene in preserving the normal physiological testis from amikacin-induced testicular injury.

MATERIALS AND METHODS

Adult male albino rats were used in this study which was provided by the Animal House of Veterinary Medicine at the University of Mosul. They aged 10–12 weeks and were weighing 250 ± 50 g. A controlled environment of humidity (45–50%), temperature (25°C ± 2°C), and lighting (12-h light, 12-h dark cycle) was applied one week before the experiment. A normal amount of water and food was supplied to the rats. The rats were given amikacin (MedoChemie®-Cyprus) and beta-carotene (PHARMAROYA company®- Turkey).

1st group served as control. The 2nd group was administered amikacin (150mg/kg/day) intraperitoneally for 14 days. 3rd group was administered both oral beta-carotene 10% (100mg/kg/day) along with intraperitoneal amikacin (150mg/kg/day) for 14 days. 4th group was

received oral beta-carotene 10% (100mg/kg/day) for 9 days as prophylaxis before giving both beta-carotene (100mg/kg/day) and amikacin (150mg/kg/day) for 14 days.

The animals were euthanized by inhaling an intensive dose of diethyl ether according to animal euthanasia regulations and sacrificed via dislocation of their cervical spine. After collecting the testis samples, they are stored in a 10% neutral buffered formalin solution which was prepared by mixing 100 mL of commercial formalin (37–40% formaldehyde) with 900 mL of distilled water [24]. All specimens were fixed for approximately 48 hours in fixative. After the removal of the paraffin wax, Hematoxyline and eosin stain was used to investigate the overall histological features of the testicular tissue. All sections were examined microscopically for the histological changes using an Olympus-CX21 light microscope while a digital microscopic camera (color USB digital image 2.0 with 9 megapixels) was utilized to get the microphotographs of various sections.

RESULTS

Control testis

The histological architecture of the testicular tissue of control showed normal seminiferous tubules with spermatogenesis cells (Figure 1). Further magnification showed also normal histological features including seminiferous tubules with spermatogonia, spermatocytes, spermatids, Sertoli cells and interstitial tissue with Leydig cells (Figure 2).

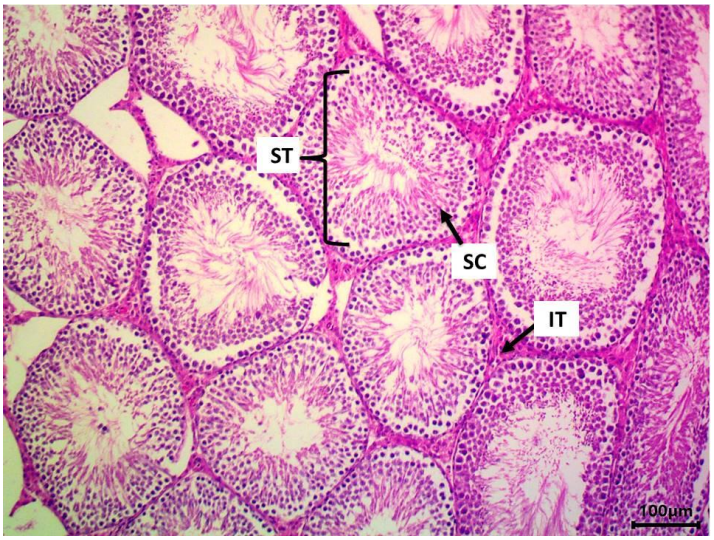

Figure 1: Photomicrograph of rat's testis of control group showing seminiferous tubules (ST) with spermatogenesis cells (ST), interstitial tissue (IT). H&E stain, Scale bar= 100µm.

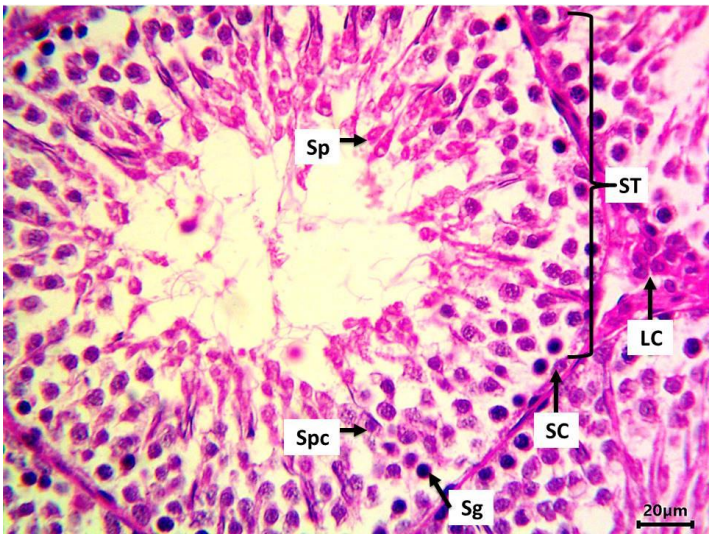

Figure 2: photomicrograph of rat's testis of control group showing seminiferous tubules (ST) with spermatogonia (Sg), spermatocytes (Spc), spermatids (Sp), Sertoli cell (Sc) and interstitial tissue (IT) with Leydig cell (Lc). H&E stain, Scale bar= 20µm.

The effect of amikacin on testis

The histological architecture of testicular tissue shows that amikacin administration caused degeneration and necrosis of cells of seminiferous tubules. In addition, atrophy and congestion of blood vessels are induced by amikacin (Figure 3,4). Further magnification showed that edema is also present between the seminiferous tubules (Figure 4).

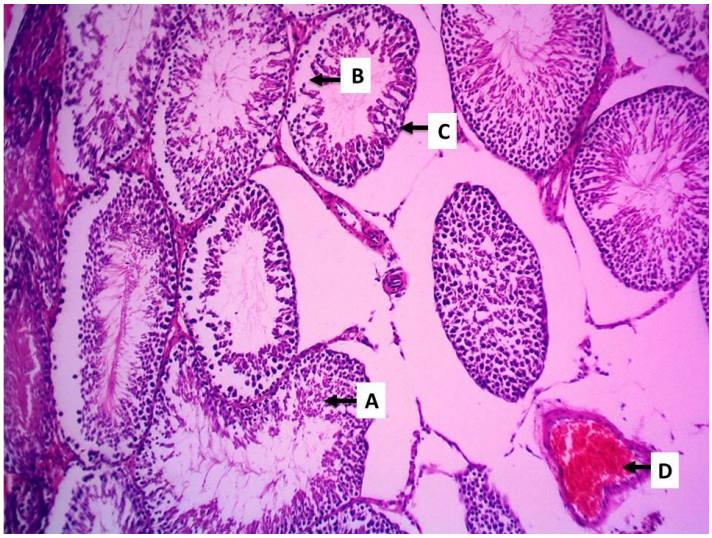

Figure 3: photomicrograph of rat testis of amikacin treated group shows degeneration (A) and necrosis (B) of cells of seminiferous tubules, with atrophy (C) and congestion of blood vessel (D). H&E stain, 100X.

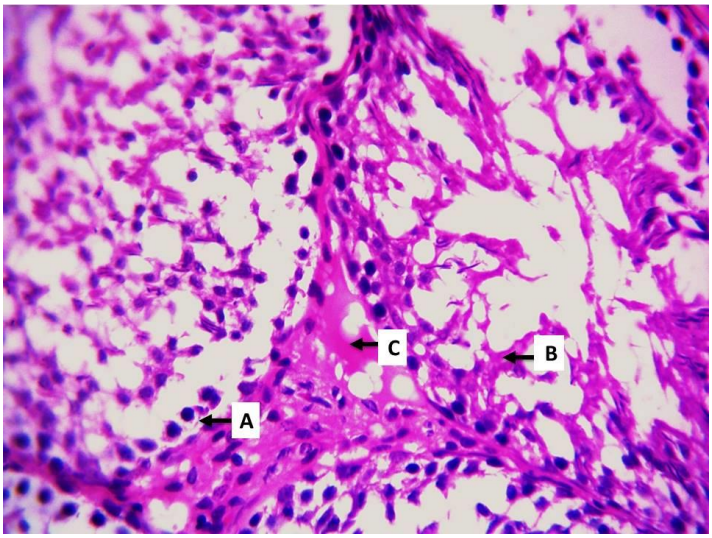

Figure 4: Photomicrograph of rat testis of amikacin treated group shows degeneration (A) and necrosis (B) of cells of seminiferous tubules and presence of edema between them (C). H&E stain, 400X.

The effect of co-administration of beta-carotene with amikacin on testis

Co-administration of both beta-carotene and amikacin resulted in an improvement in the testicular tissue in comparison with an only-amikacin group. However, there was no complete preservation of the testis in that mild degeneration of cells of the seminiferous tubules and edema were still present (Figure 5,6).

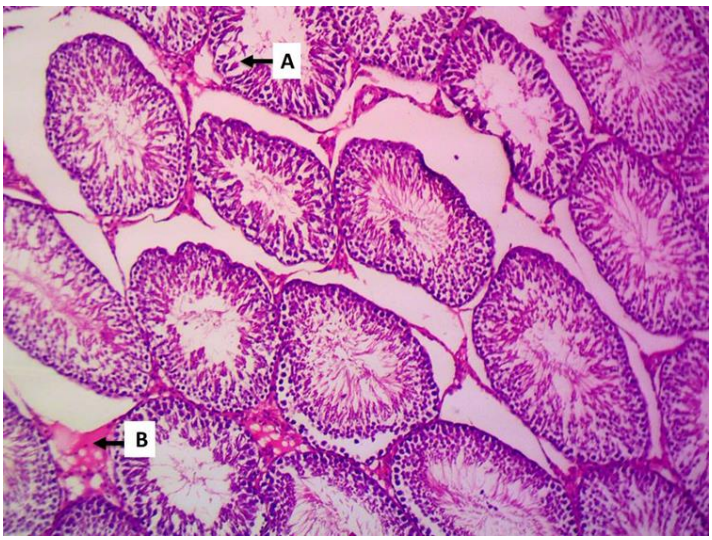

Figure 5: Photomicrograph of rat testis of amikacin with beta-carotene treated group shows mild degeneration of cells of seminiferous tubules, with edema between them (B). H&E stain, 100X.

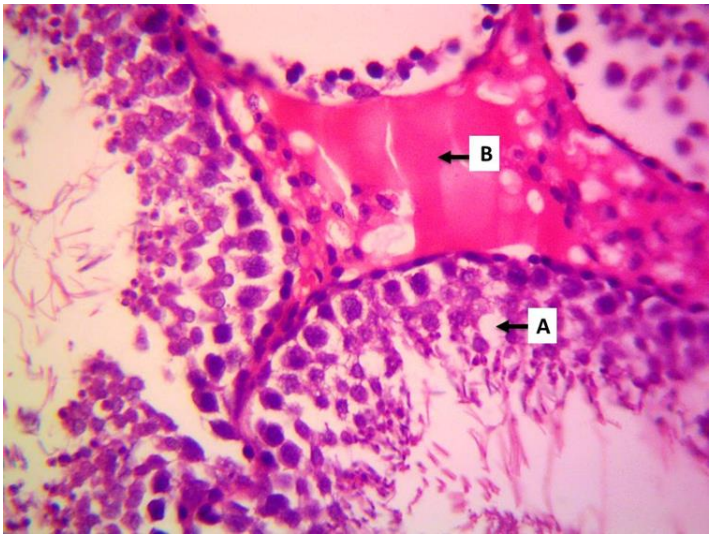

Figure 6: Photomicrograph of rat testis of amikacin with beta-carotene treated group shows mild degeneration of cells of seminiferous tubules, with edema between them (B). H&E stain, 100X.

The effect of administration of beta-carotene as prophylaxis on testis

Administration of beta-carotene as prophylaxis before co-administration of both beta-carotene and amikacin showed complete protection and preservation of the testicular tissue against amikacin's adverse effect. Histological sections showed normal architecture of the testis which are represented by physiological seminiferous tubules with spermatogenic cells (Figure 7,8).

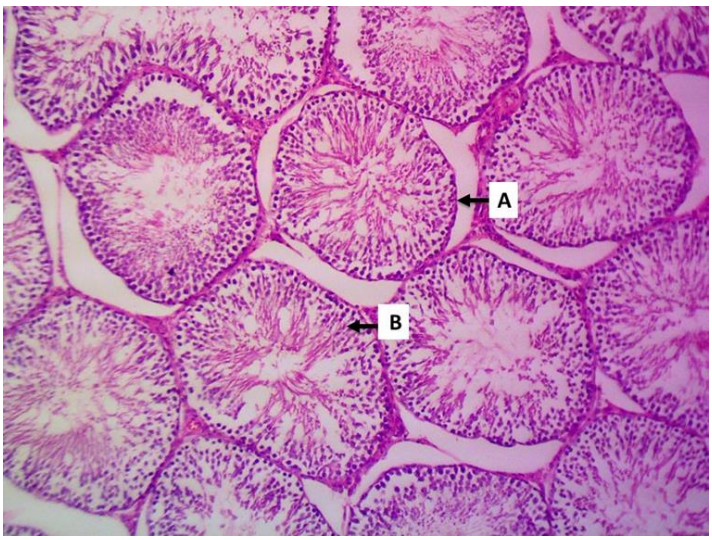

Figure 7: Photomicrograph of rat testis of beta-carotene administration then amikacin with beta-carotene treated group shows normal architecture represented by seminiferous tubules (A) with spermatogenic cells (B). H&E stain, 100X.

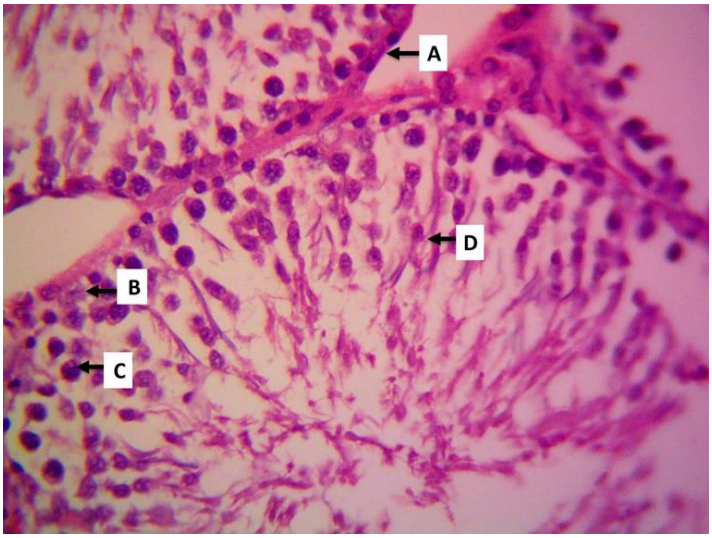

Figure 8: Photomicrograph of rat testis of beta-carotene administration then amikacin with beta-carotene treated group shows normal architecture representing by seminiferous tubules (A) with Sertoli cells (B), spermatocytes (C) and spermatids (D). H&E stain, 400X.

DISCUSSION

Several researchers discussed the most common adverse effect of amikacin which are ototoxicity [25] and nephrotoxicity [26]. In addition, many studies investigated the methods of protection against this disadvantageous effect of amikacin on the ear [27,28] and kidney [29,30]. In contrast, little information is known about the adverse effect of amikacin on testicular physiology. In addition, it is important to understand the ways of preservation of the testicular histology and, consequently, physiology from the deleterious effect of the medication.

Concerning testes, only recent research by Elsawah et al, (2020) has studied the effects of amikacin on testicular tissue and function in male Wistar rats. They have found that amikacin can adversely affect fertility status by affecting sperm parameters and the level of gonadotropins. Additionally, amikacin has resulted in abnormalities in the histological architecture of the testis [31]. In line with this previous study, the present study showed also a deleterious effect of amikacin on the testis in that the drug caused a range of histopathological changes including degeneration and necrosis of cells of seminiferous tubules, with atrophy and congestion of blood vessels. The dose used in this study (150mg/kg/day) was high enough to cause these histopathological changes. Elsawah et al., (2020) found that the effect of aminoglycosides is dose-dependent and a testicular adverse effect can be noticed even at the therapeutic dose (54.75mg/kg/day). Other aminoglycosides are known to have a negative impact on fertility status via decreasing sperm count, motility, and viability. These aminoglycosides include streptomycin, gentamicin, and neomycin In addition, these aminoglycosides can decrease the weight of the testis, seminal vesicle, and epididymis [32].

Interestingly, the present study investigated the way of preservation of the testicular tissue from this amikacin's effect via co-administration of beta-carotene as an antioxidant. Beta-carotene has a powerful antioxidant activity which is summarized by a (Mueller and Boehm 2011) review [33]. The present study showed that co-administration of bet-carotene along with amikacin inhibited partially the histopathological features induced by the latter drug. To the best of our knowledge,

this is the first time to highlight this novel and significant protective effect of beta-carotene against amikacin-induced testicular toxicity. This protective effect of beta-carotene is identified against testicular injury induced by other drugs. Beta-carotene can decrease the side effects of methotrexate via decreasing apoptotic cell death [34]. Additionally, another study showed protective antioxidant activity against cadmium-induced testicular toxicity [35]. In addition, the administration of beta-carotene as prophylaxis before amikacin's administration caused complete protection against the damaging effect of amikacin on the testis. This prophylactic role of beta-carotene is in line with another study by Abd-El Azeim et al, (2012) who found that the pre-treatment with the prophylactic dose of beta-carotene was enough to prevent the damaging effect of acrylonitrile on testicular tissue and function [36].

In summary, a high dose of amikacin can adversely affect the testis. Administration of beta-carotene can alleviate this testicular effect either partially by giving both beta-carotene and amikacin at the same time or completely by giving beta-carotene as prophylaxis before amikacin administration. It is recommended to monitor and control the fertility status during the application of this drug especially if it is used in high doses or after a long duration of treatment. Beta-carotene is also recommended to be given as prophylaxis in such conditions to protect the normal histology and, consequently, the physiology of the testis.

CONFLICT OF INTEREST

None.

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