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Protective and ameliorative effects of Curcumin and/or Quercetin against gentamicin induced testicular damage in rats

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Abstract

The aminoglycoside Gentamicin is a commonly used antibiotic counteracting the Gram-ve microorganisms. Rats administered with Gentamicin showing a reduced testicular weight and inhibited spermatogenesis, as gentamicin generates ROS, decreasing the antioxidant reserve and accelerate mitochondrial dysfunction which then leads to apoptosis and testicular tissue destruction. This study was designed to investigate the protective effects of curcumin and/or quercetin on the gentamicin induced testicular damage or toxicity in sexually mature adult rats. Pre-treatment with curcumin and/or quercetin, markedly inhibited and ameliorated the reduction in sperm count, viability, motility and sperm production in gentamicin treated rats. Moreover, curcumin and/or quercetin, significantly reduce teratospermia including head or tail abnormalities that observed in the gentamicin treated rats. These abnormalities were effectively normalized by curcumin and/or quercetin pretreatment improving the testicular tissue via counteracting of ROS, improvement of spermatogenesis and ameliorate the sperms quality and quantity. In conclusion supplementation of curcumin and/or quercetin improving the sperm count and morphology via testicular cell repair, counteracting the undesirable effect of gentamicin.

Keywords: Gentamicin, Curcumin, Quercetin, ROS,

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Introduction

Aminoglycosides are one of the main bactericidal antibiotic classes commonly used for different types of infection e.g. urinary tract infection, infective endocarditis and blood stream infection (Mermel et al., 2009; Gupta et al., 2010; Habib et al., 2015). Aminoglycosides are broad-spectrum antibiotics that act through inhibition of protein synthesis (Davis, 1987). The 30S subunit of the bacterial ribosome is bound by gentamicin, a bactericidal antibiotic, which inhibits protein synthesis. Eliminating the ribosome's capacity to distinguish between appropriate messenger RNA and transfer RNA interactions is the main mechanism of action (Drugbank, 2013). Moreover, that antibiotic induces testicular damage and toxicity plus its well-known nephrotoxic side-effects. Studies have proven that gentamicin can impair sperm count, motility, shape, reduce testicular weights and cause apoptosis via mitochondrial dysfunction in the testis, resulting in testicular damage and dysfunction (Khaki *et al*., 2008; Nouri *et al*., 2009; Zahedi *et al*., 2010).The mechanism of gentamicin-induced testicular damage is still not completely clarified, but the generation of toxic ROS have been linked to the pathological effects of gentamicicn toxicity (Pedraza-Chaverrí et al., 2004; Hong et al., 2006). Furthermore ROS plays a vital role in the reproductive disorders pathogenesis; impaired sperm function and induced male infertility. (Sikka, 1996; Kothari et al., 2010; Kim et al., 2012). Turmeric's (Curcuma longa) rhizome contains a yellow colour component called curcumin (CUR), which has several pharmacological and biological properties, such as anti-inflammatory, anti-carcinogenesis, and antioxidant properties. Recent research has showed that CUR has anti-apoptotic properties and inhibits the death of rat thymocytes caused by toxic dexamethasone and the death of breast cancer cells caused by chemotherapy (singh, 2007; Zhang et al., 2013). CUR stops UV irradiation's apoptotic effects, via inhibition of the cytochrome C release from the mitochondria, reducing the loss of mitochondrial membrane potential, and a diminished ROS generation. Some researches show that CUR has benefits for testicular injury (Naz, 2014; Noorafshan et al., 2014). One of the most prevalent dietary polyphenolic chemicals, quercetin (3, 3′, 4′, 5, 7-pentahydroxyflavone) is heavily contained in red wine, tea, and onions (Formica & Regelson, 1995). The anti-atherogenic, anti-inflammatory, anticoagulant, anti-hypertensive, and anticarcinogenic effects of quercetin have been demonstrated (Kris-Etherton et al., 2004; Williams et al., 2004). There are conflicting findings on quercetin's effects on the male reproductive system (Mi et al., 2010; Bu et al., 2011). According to recent studies, quercetin is utilised as a substitute medication to treat male infertility (Taepongsorat et al., 2008). Our manuscript clarified the ameliorative or the anti-ROS effect of curcumin and/or quercetin against gentamicin induced testicular damage, which in turn improving testicular tissue and male fertility concluded in the normal sperm count, viability, motility and morphology.

Materials and methods

Drugs

Gentamicin

It was purchased from Memphis Co. for Pharm. And Chem. Ind. (MEMCO), Egypt; under authority of Schering-Plough Corporation, U.S.A, Cairo, Egypt. In the form of ampoules of 2 ml 40 mg/mL of

gentamicin sulphate. The therapeutic dosewas 100 mg/kg b.wt injected I/P as reported Muhammet et al., (2018).

Curcumin

Curcumin purchased from Sigma Chemical, St. Louis, MO, USA. The oral dose of curcumin was 100 mg/kg b.wt as reported by **(**Mehmet et al**, 2012).**

Quercetin

It was purchased from Sigma Chemical, St. Louis, MO, USA. The intra peritoneal dose of quercetin was 20 mg/kg b.wt according to Ranawat et al., (2012).

Animals

Twenty five sexually mature male rats of Sprague Dawley strain weighing 180 - 200 g body weight and 12 - 14 weeks old were used in this study. Rats purchased from Laboratory Animal Colony, Helwan, Egypt. Rats were housed under controlled temperature at 23 ± 1ºC, humidity at 52 % and 12-hr light/12-hr dark schedule. Animals were fed on commercial rat pellets manufactured by Cairo Agriculture Development Company, The 6th October City, Egypt and tape water was provided ad libitum. The animals were left 15 days for acclimatization before the beginning of the experiment. Animals were admitted in the department of biochemistry at Faculty of Veterinary Medicine, South Valley University. Following the ethical consideration of experimental animals of South Valley University. The Ethics Committee of the Faculty of Veterinary Medicine, South Valley University approved the use of experimental animals and the study design (Approval no.78/2022).

Experimental design

Rats were distributed into 8 equal groups of 5 animals each. Group (1) used as control group received physiological saline solution daily for 6 days. Group (2): received DMSO daily for 6 days. Group (3): received curcumin daily for 6 days. Group (4) received quercetin daily for 6 days. Group (5): received gentamicin daily for 6 days Group (6): received curcumin and also received gentamicin daily for 6 days. Group (7): received quercetin and also received gentamicin daily for 6 days. Group (8): received curcumin, quercetin and also received gentamicin daily for 6 days. The experiment lasted for 6 days. The intra peritoneal dose of gentamicin was 100 mg/kg b.wt as reported by hamoud et al., (2019). The oral dose of curcumin was 100 mg/kg b.wt as reported by Mehmet et al., (2012).The intra peritoneal dose of quercetin was 20 mg/kg b.wt according to Ranawat et al., (2012). Rats were then anesthetized and semen samples were collected from cauda epididymis for semen analysis.

Semen analysis

The semen in epididymis was obtained by cutting of cauda epididymis using surgical blades and squeezed in a sterile dry Petri dish. The semen content was diluted 25 times with 0.9% physiological saline (Kempinas et al, 1998) and thoroughly mixed to estimate percentage of sperm progressive motility and sperm count (WHO, 2010). Thereafter, one drop of sperm suspension was withdrawn, smeared on a glass slide and stained by Eosin-Nigrosin. The stained seminal smears were examined microscopically to determine percentage of sperm viability (ratio of live/dead sperms) (Amann, 1982). The Diff-Quik® is rapid methods for evaluating sperm morphology (Sousa et al. 2009) and involve fixation followed by staining with two staining solutions.

Statistical analysis

Data were presented as means± SE. Statistical analysis between different

experimental groups was carried out using a one-way analysis of variance (ANOVA)test followed by Duncan's multiple range tests] using computerized SPSS (Statistical Program of Social Sciences, Version 15, Chicago) program. Differences were considered significant at $P<0.05$.

Results

The results illustrated in **Fig. (1, 2, 3, 4, 5 and 6)** distinguished the effect of I.P injection of gentamicin at therapeutic doses (100mg/kg b.wt./week) with and without curcumin (100mg/kg b.wt./day orally) and quercetin (20mg/kg b.wt./day orally) for 6 successive days on semen analysis parameters including {Sperm motility%, Sperm vitality %, Normal sperm morphology% and Sperm count}. Treatment of rats with gentamicin induced significant decrease in (Sperm motility %, Sperm vitality %, Normal sperm morphology % and Sperm count). In contrast, treatment with either curcumin and or quercetin significantly improved all the semen analysis parameters when compared with gentamicin treated group. The changes in the sperm motility%, sperm morphology%, sperm count and sperm vitality% were non-significantly different between curcumin groups and quercetin treated groups.

Abnormalities of sperm morphology:

I.P injection of gentamicin induced abnormalities in the sperm cells shape including 1) abnormal sperm head 2) bent sperm tail and 3) detached sperm tail. In addition to 4) combined sperm abnormalities. The morphological changes induced by gentamicin inoculation returned to normal after treatment with curcumin and quercetin.

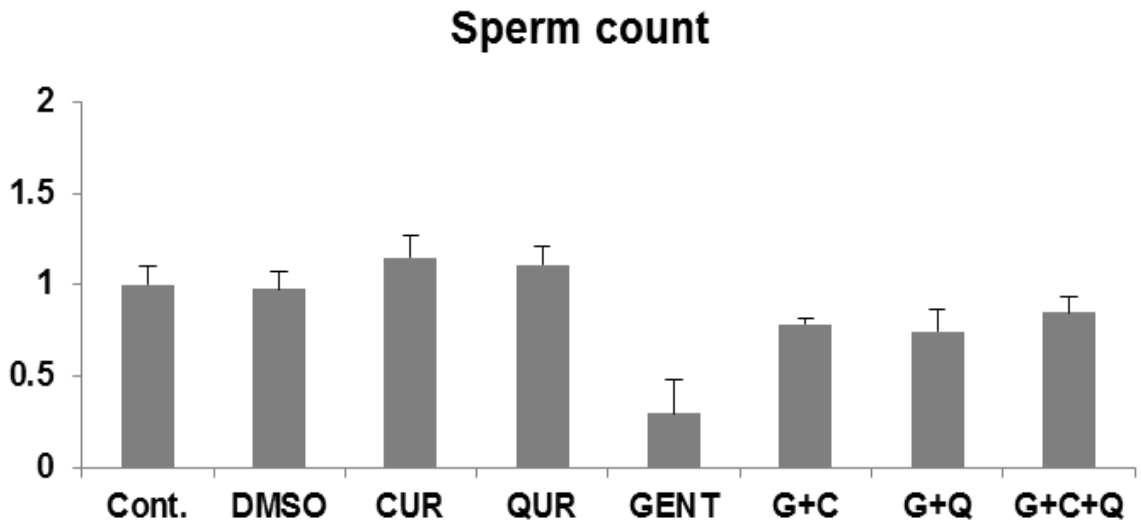


Fig. 1: The effect of I.P injection of gentamicin at therapeutic doses (100mg/kg b.wt./day) with and without curcumin (100mg/kg b.wt./day orally) and quercetin (20mg/kg b.wt./day orally) for 6 successive days on sperm count% in male albino rat. (M ± S.E) (N= 5).

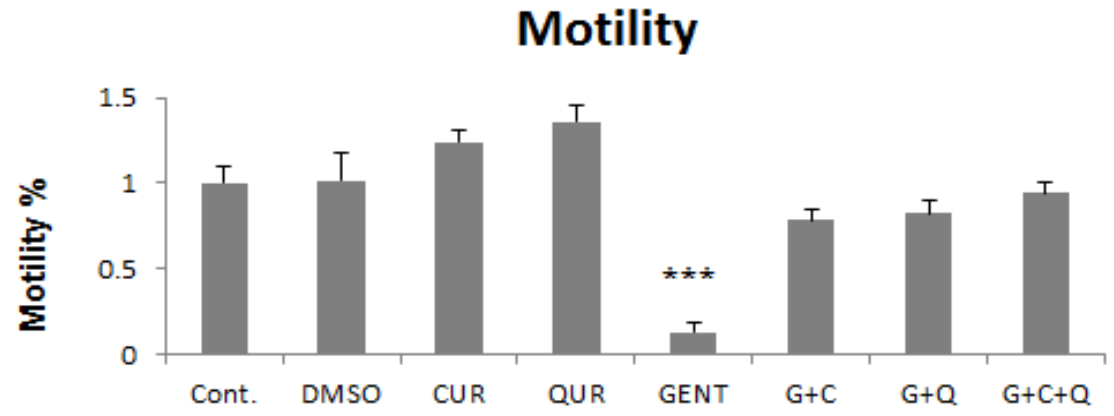
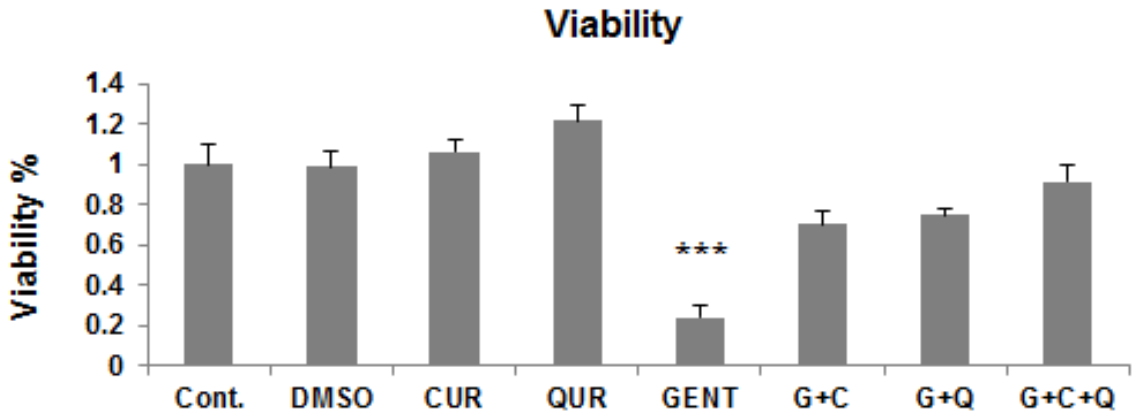
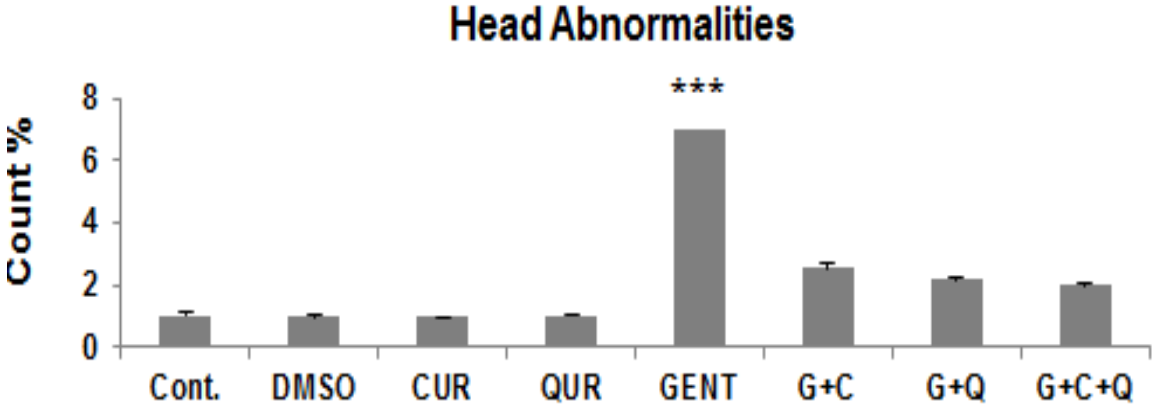


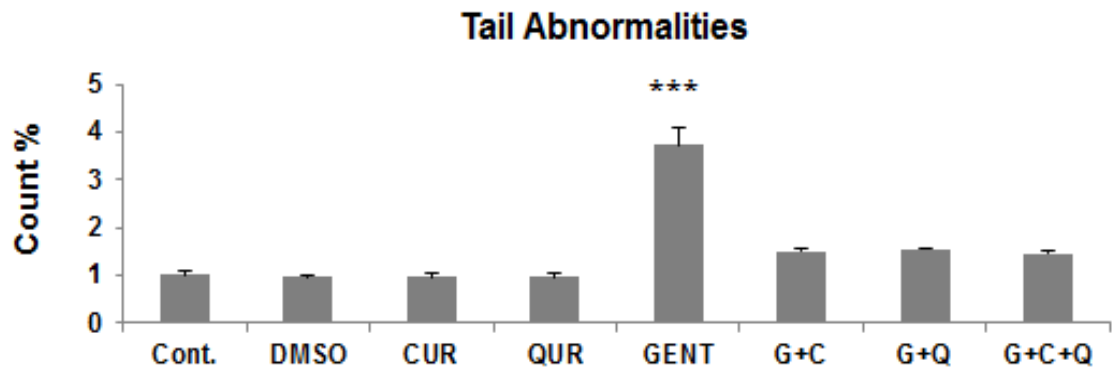
Fig. 2: The effect of I.P injection of gentamicin at therapeutic doses (100mg/kg b.wt./day) with and without curcumin (100mg/kg b.wt./day orally) and quercetin (20mg/kg b.wt./day orally) for 6 successive days on sperm motility% in male albino rat. (M ± S.E) (N= 5).



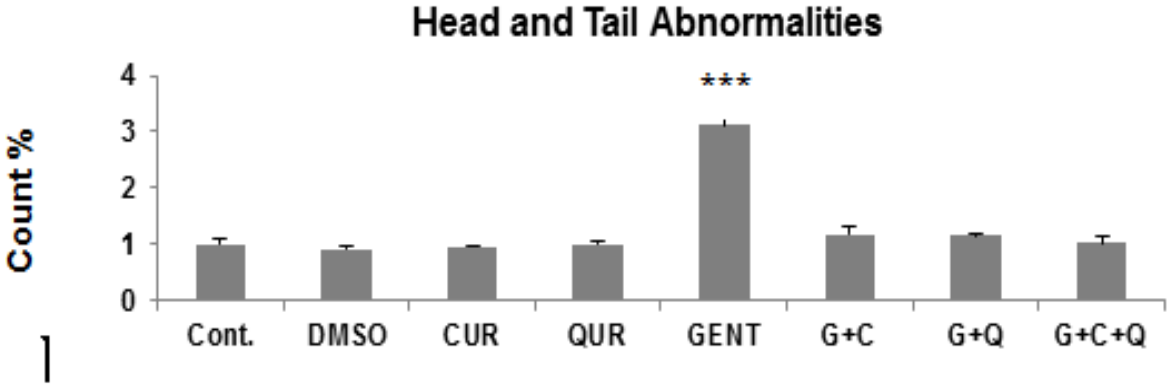
Fig(3): The effect of I.P injection of gentamicin at therapeutic doses (100mg/kg b.wt./day) with and without curcumin (100mg/kg b.wt./day orally) and quercetin (20mg/kg b.wt./day orally) for 6 successive days on sperm viability% in male albino rat. (M ± S.E) (N= 5).



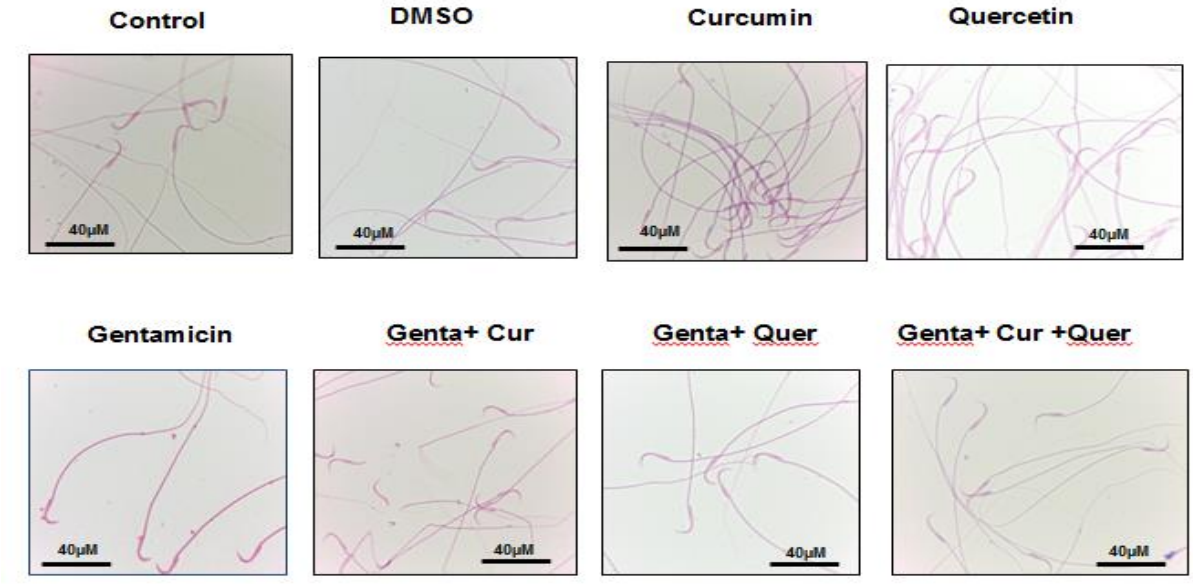
Fig(4): The effect of I.P injection of gentamicin at therapeutic doses (100mg/kg b.wt./day) with and without curcumin (100mg/kg b.wt./day orally) and quercetin (20mg/kg b.wt./day orally) for 6 successive days on head abnormalities% in male albino rat. (M ± S.E) (N= 5).



Fig(5): The effect of I.P injection of gentamicin at therapeutic doses (100mg/kg b.wt./day) with and without curcumin (100mg/kg b.wt./day orally) and quercetin (20mg/kg b.wt./day orally) for 6 successive days on tail abnormalities% in male albino rat. (M ± S.E) (N= 5).



Fig(6): The effect of I.P injection of gentamicin at therapeutic doses (100mg/kg b.wt./day) with and without curcumin (100mg/kg b.wt./day orally) and quercetin (20mg/kg b.wt./day orally) for 6 successive days on head and tail abnormalities% in male albino rat. (M ± S.E) (N= 5).



Fig(7): microscopically examined sperm showing the effect of I.P injection of gentamicin at therapeutic doses (100mg/kg b.wt./day) with and without curcumin (100mg/kg b.wt./day orally) and quercetin (20mg/kg b.wt./day orally) for 6 successive days.

Discussion

In the current study, gentamicin had undesirable effect on normal sperms parameters including sperm motility, viability, count and morphology. These findings are in agreement with the findings of Kim et al, 2013. Epididymal sperm count and sperm motility in the gentamicin group were significantly decreased in comparison with those of the control group. A significant decrease in serum testosterone, testicular testosterone and estradiol, sperm counts, sperm viability and an increase in ratio of fragmented DNA (Mohamed *et al*., 2017). Germ cell apoptosis was caused by gentamicin's creation of an oxidative stress-status in the testis by boosting the production of free radicals and lipid peroxidation followed by hypo-spermatogenesis (Turner and Lysiak, 2008). Gentamicin significantly decreased the epididymis, testes and seminal vesicles weights as well the sperm count and motility as a result of Leydig and spermatogenic cells destruction (Khaki, 2015).

According to Moretti et al. (2012), quercetin is effective at low concentrations and has a limited impact on sperm motility and viability, but it has been shown to reduce lipid peroxidation which may help to partially support the results of our investigation. Quercetin has significant beneficial effects on the sperm viability, motility, and serum total testosterone and could be effective for maintaining healthy sperm parameters and male reproductive function (Khaki, 2010).

The use of turmeric and curcumin appeared to be effective in reducing nitrate-induced reproductive changes, as evidenced by normalized NO, lipid peroxidation, protein carbonyl and lipid profile, as well as antioxidant components, total protein, DNA, RNA, male hormones and sperm number. The results thus suggested that tumeric and curcumin could be useful in treatment of male infertility, with oligospermia, reduced male sex hormones and other adverse reproductive outcomes (El-Wakf, 2011). Curcumin's ability to shield cells from oxidative stress in spermatogenic cells of seminiferous tubules and Leydig cells of the stroma may account for its protective impact on the testis (Aly et al., 2009). It has been shown that curcumin protects by controlling lipid peroxidation and boosting the antioxidant defence system (Kalpana and Menon, 2004). The protective effect of curcumin was explicitly linked to its free radical scavenging activity, stimulation of detoxification enzymes, and prevention of degenerative illnesses (Manikandana et al., 2004). More specifically, curcumin drastically decreased the levels of free radicals.

Conclusion:

The results of this work demonstrated that IP administration of gentamicin daily for six days induced deleterious changes in tests, it could be concluded that use of genamicin induce hazardous effects in dose and time dependent especially in male animals mainly that used for breeding. It can be concluded that use of curcumin and quercetin have the capability to alleviate many of the harmful effects of gentamicin.

Declaration of Competing Interest:

There are no funding or competing interests to this report.

References

Aly H. (2019). Testicular toxicity of gentamicin in adult rats: Ameliorative effect of lycopene. Human and Experimental Toxicology. 38(11): 1302–1313

Amann RP (1982). Use of animal models for detecting specific alterations in reproduction. *Fundamental and Applied Toxicology*, *2*(1): 13-26.

Bu T, Mi Y, Zeng W and Zhang C (2011). Protective effect of quercetin on cadmium-induced oxidative toxicity on germ cells in male mice. *The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology*, *294*(3): 520-526.

Davis BD (1987). Mechanism of bactericidal action of aminoglycosides. *Microbiological reviews*, *51*(3): 341-350.

El-Wakf AM, Elhabiby EM, El-kholy WM and El-Ghany EA(2011). Use of tumeric and curcumin to alleviate adverse reproductive outcomes of water: Nitrate pollution in male rats. *Nat Sci*, *9*: 229-239.

Formica JV and Regelson W (1995). Review of the biology of quercetin and related bioflavonoids. *Food and chemical toxicology*, *33*(12): 1061-1080.

Gupta K, Hooton T M, Naber KG, Wullt B, Colgan R, Miller LG and Soper DE (2011). International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clinical infectious diseases*, *52*(5): e103-e120.

Habib G, Lancellotti P, Antunes MJ, Bongiorni MG, Casalta JP, Del Zotti F and Zamorano JL (2015). 2015 ESC guidelines for the management of infective endocarditis: the task force for the management of infective endocarditis of the European Society of Cardiology (ESC) endorsed by: European Association for Cardio-Thoracic Surgery (EACTS), the European Association of Nuclear Medicine (EANM). *European heart journal*, *36*(44): 3075-3128.

Hamoud AEMM (2019). Possible Role of Selenium Nano-particles on Gentamicin-Induced Toxicity in Rat Testis:Morphological and Morphometric study: The Egyption journal of Histology , 42(4): 861-873.

Hong SH, Park SK, Cho YS, Lee HS, Kim KR, Kim MG, Chung WH (2006). Gentamicin induced nitric oxide-related oxidative damages on vestibular afferents in the guinea pig. Hear Res 211:46–53.

Kalpana C, Menon V (2004): Protective effect of curcumin on circulatory lipid peroxidation and antioxidant status during nicotine-induced toxicity. Toxicology Mechanisms and Methods14:339-343

Kanter M, Aktas C and Erboga M (2013). Curcumin attenuates testicular damage, apoptotic germ cell death, and oxidative stress in streptozotocin-induced diabetic rats. *Molecular nutrition & food research*, *57*(9): 1578-1585.

Kempinas WD, Suarez JD, Roberts NL, Strader L, Ferrell J, Goldman JM and Klinefelter GR (1998). Rat epididymal sperm quantity, quality, and transit time after guanethidine-induced sympathectomy. Biol Reprod, 59 (4):890-896.

Khaki A (2015). Assessment on the adverse effects of Aminoglycosides and Flouroquinolone on sperm parameters and male reproductive tissue: A systematic review. *Iranian journal of reproductive medicine*, *13*(3): 125.

Khaki A, Fathiazad F, Nouri M, Khaki A, Maleki NA, Khamnei HJ and Ahmadi P (2010). Beneficial effects of quercetin on sperm parameters in streptozotocin-induced diabetic male rats. *Phytotherapy Research*, *24*(9): 1285-1291.

Khaki A, Novin MG, Khaki AA, Nouri M, Sanati E, Nikmanesh M (2008). Comparative study of the effects of gentamicin, neomycin, streptomycin and ofloxacin antibiotics on sperm parameters and testis apoptosis in rats. Pak J Biol Sci 11:1683–1689

Kim SH, Lee IC, Lim JH, Moon C, Bae CS, Kim SH, Shin DH, Kim HC, Kim JC (2012). Spermatotoxic effects of a-chlorohydrin in rats. Lab Anim Res 28:11–16.

Kim SH, Lee IC, Baek HS, Shin IS, Moon C, Kim SH and Kim JC (2014). Melatonin prevents gentamicin-induced testicular toxicity and oxidative stress in rats. *Andrologia*, *46*(9): 1032-1040.

Kris-Etherton PM, Lefevre M, Beecher GR, Gross MD, Keen CL and Etherton TD (2004). Bioactive Compounds In Nuitrion And Health-Research Methodologies For Establishing Biological Function: The Antioxidant and Anti-inflammatory effects of Flabonoids on Atherosclerosis. *Annual review of nutrition*, *24*: 511.

Kothari S, Thompson A, Agarwal A, du Plessis SS (2010). Free radicals: their beneficial and detrimental effects on sperm function. Indian J Exp Biol 48:425–435.

Manikandana P, Sumitraa M, Aishwaryaa S, Manohar BM, Lokanadam B, Puvanakrishnana R (2004). Curcumin modulates free radical quenching in myocardial ischaemia in rats The International Journal of Biochemistry & Cell Biology. 36 (10): 1967-1980.

Mermel LA, Allon M, Bouza E, Craven DE, Flynn P, O'Grady NP and Warren DK (2009). Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection. *Clinical infectious diseases*, *49*(1): 1-45.

Mi Y, Zhang C, Li C, Taneda S, Watanabe G, Suzuki AK, Taya K (2010). Quercetin attenuates oxidative damage induced by treatment ofembryonic chicken spermatogonial cells with 4-nitro-3-phenylphenol in dieselexhaust particles. Biosci Biotechnol Biochem, 74:934–938.

Mohamed SM, Mohamed SF, Hashim NI, Saleh SY, Bakeer MR and Sawiress FA (2017). Effect of stem cell therapy on gentamicin induced testicular dysfunction in rats. *Journal of Health and Medical Informatics*, *8*(3).

Moretti E, Mazzi L,Bonechi C, , Collodel G(2012). Effect of quercetin, rutin, naringenin and epicatechin on lipid peroxidation induced in human sperm. Reproductive Toxicology 34(4).

Muhammet k, Budak H and Çiftci M(2018). Amoxicillin and gentamicin antibiotics treatment adversely influence the fertility and morphology through decreasing the Dazl gene expression level and increasing the oxidative stress. *Archives of physiology and biochemistry*, *125*(5): 447-455.

Naz RK (2014). The effect of curcumin on intracellular pH (pHi), membrane hyperpolarization and sperm motility. *Journal of Reproduction & Infertility*, *15*(2): 62.

Noorafshan A, Abdollahifar MA, Asadi-Golshan R, Rashidian-Rashidabadi A and Karbalay-Doust S (2014). Curcumin and sertraline prevent the reduction of the number of neurons and glial cells and the volume of rats' medial prefrontal cortex induced by stress. *Acta Neurobiol Exp (Wars)*, *74*(1): 44-53.

Nouri M, Khaki A, Azar FF, Rashidi MR (2009). The protective effects of carrot seed extract on spermatogenesis and cauda epididymal sperm reserves in gentamicin treated rats. Yakhte Med J 11:327–333.

Pedraza-Chaverrı J, Barrera D, Maldonado PD, ChirinoYI,Macıas-Ruvalcaba NA, Medina-Campos ON, Castro L, Salcedo MI, Hernandez-Pando R (2004). S-allylmercaptocysteine scavenges hydroxyl radical and singlet oxygen in vitro and attenuates gentamicin-induced oxidative and nitrosative stress and renal damage in vivo. BMC Clin Pharmacol 4:5.

Ranawat P, Kaushik G, Saikia UN, Pathak CM and Khanduja KL (2013). Quercetin impairs the reproductive potential of male mice. *Andrologia*, *45*(1): 56-65.

Sikka SC (1996). Oxidative stress and role of antioxidants in normal and abnormal sperm function. Front Biosci 1:78–86.
Singh S (2007). From exotic spice to modern drug?. *Cell*, *130*(5): 765-768.

Sousa APM, Tavares RS, De la Calle JFV, Figueiredo H, Almeida V, Almeida-Santos T and Ramalho-Santos J (2009) Dual use of Diff-Quik

like stains for the simultaneous evaluation of human sperm morphology and chromatinstatus. Hum. Reprod. 24: 28-36.

Taepongsorat L, Tangpraprutgul P, Kitana N and Malaivijitnond S (2008). Stimulating effects of quercetin on sperm quality and reproductive organs in adult male rats. *Asian journal of andrology*, *10*(2): 249-258.

Turner TT and Lysiak JJ (2008). Oxidative stress: a common factor in testicular dysfunction. *Journal of andrology*, *29*(5): 488-498.

WHO (2010): World Health Organization laboratory manual for the examination and processing of human semen. WHO Press: Geneva.

Williams RJ, Spencer JP, Rice-Evans C (2004). Flavonoids: antioxidants or signaling molecules? Free Radic Biol Med, 36:838–849.

Zahedi A, Khaki A, Ahmadi-Ashtiani HR, Rastegar H, Rezazadeh S (2010). Zingiber officinale protective effects on gentamicin's toxicity on sperm in rats. J Medici Plant 9: 93–98

Zhang Y, Cao H, Yu Z, Peng HY and Zhang CJ (2013). Curcumin inhibits endometriosis endometrial cells by reducing estradiol production. *Iranian journal of reproductive medicine*, *11*(5): 41