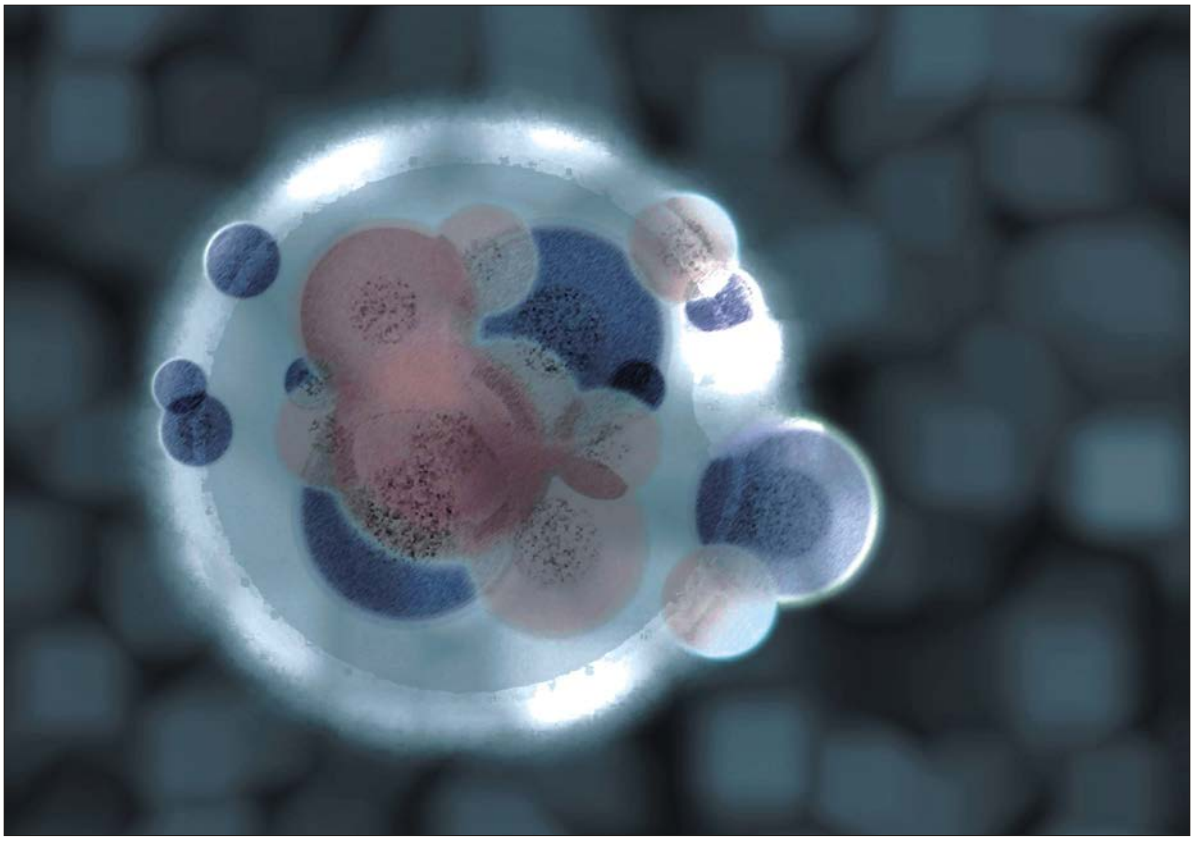

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Impact of α-tocopherol on Metronidazole and Tetracycline-induced Alterations in Reproductive Activities of Male Albino Rats

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Abstract: Antimicrobial drugs have been reported to have adverse effects on male fertility. The present study reports the role of α- tocopherol on metronidazole and tetracycline induced reproductive alterations in albino rats. Male albino rats (5/group) were treated with 20 mg kg^{-1} bw day^{-1} metronidazole or 60 mg kg^{-1} day^{-1} tetracycline with or without 15 mg kg^{-1} bw α-tocopherol for 8 weeks. The reversibility of effects after 4 weeks recovery period was determined in separate groups of 5 rats. The control groups received distilled water (vehicle) and 15 mg kg^{-1} day^{-1} α- tocopherol for 8 weeks. Metronidazole and tetracycline significantly ($p<0.05$) reduced the weight of the epididymis, sperm count, motility and serum testosterone levels and increased the activity of endogenous superoxide dismutase (SOD) in the testis. Alpha-tocopherol significantly ($p<0.05$) decreased the weight of the testis, epididymis, sperm motility and serum testosterone levels. Co-administration of metronidazole or tetracycline with α-tocopherol caused significant restoration in sperm indices and SOD activity while it produced no effect on testosterone secretion. The results suggest that the effects of metronidazole and tetracycline on male reproductive functions, which are partially reversible, could be mediated via a reduction in serum testosterone level and probably also via the free radical generating mechanism.

Key words: Antimicrobial, antioxidant, reproduction, male, rat

INTRODUCTION

Approximately 10-15% of couples demonstrate primary infertility and of these a male factor is identified in approximately 50% of cases (Kolettis, 2003). Many extrinsic and environmental factors including the increased used of antibiotics have been implicated as potential causes of male infertility (Nelson and Bunge, 1974; Schelegel *et al*., 1991). Oral antimicrobial agents belonging to the beta-lactams, quinolones, macrolides, tetracyclines and the trimethoprim-sulfamethaxazole combination are among the most prescribed classes of drugs in medical practice (Cerny, 1996). A knowledge of the potential side effects considered in the light of various patient associated factors such as genetic make-up, renal and liver function, underlying diseases, drug allergies and co-administered drugs, is important in order to minimize the risk of adverse reactions. The side effects of antibiotics on spermatogenesis are seldom discussed in the literature. The increase incidence of sterility in man along with the increase in number of new therapeutic drugs administered to patients has made experimental work on antibiotics in male reproduction important. Antibiotics have been found to adversely affect male reproductive functions (Timmermans, 1974; Schlegel *et al*., 1991; Hargreaves *et al*., 1998). Recently we reported the adverse impact of ampicillin, cloxacillin and tetracycline on *in vivo* and *in vitro* male reproductive functions (Raji *et al*., 2006; Awobajo *et al*., 2006). These studies revealed that ampicillin, cloxacillin and tetracycline significantly reduced sperm count, motility, viability and morphologically normal spermatozoa and testosterone secretion.

However the mechanism (s) by which antibiotics exert their antifertility effect in the male are yet to be fully elucidated. Several studies have demonstrated the antifertility effects of free radicals and numerous mechanisms of action have been proposed (Peltola *et al*., Punta *et al*., 1996; Giannattasio *et al*., 1997). Previous investigations suggest that oxidative stress may be an

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important mediator of testicular injury. The present study was therefore designed to investigate the role of an antioxidant, α-tocopherol, on metronidazole (antiprotozoal) and tetracycline (antibiotic) induced alterations in male reproductive functions.

MATERIALS AND METHODS

Animals and drugs: Male Wistar strain albino rats weighing 190-240 g obtained from the National Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria, were used for the study. Rat cubes (Ladokun Feeds, Ibadan, Nigeria Ltd.) and water were provided *ad libitum*. The rats were divided into groups and each group was housed separately in a wire mesh cage. Metronidazole, tetracycline formulations and α-tocopherol obtained from the University of Ibadan Health Centre were used for the study.

Experimental design: Male albino (5/group) rats were randomly allotted into experimental groups as follows. Group1 rats (negative control) were administered with distilled water (vehicle for the drug). Group II rats, which also served as the positive control, were treated with 15 mg kg^{-1} bw of α-tocopherol. Group III rats were treated with 20 mg kg^{-1} bw metronidazole and group IV rats received 20 mg kg^{-1} bw metronidazole plus 15 mg kg^{-1} bw α-tocopherol. Group V and VI rats were treated with 60 mg kg^{-1} bw tetracycline and 60 mg kg^{-1} bw tetracycline plus 15 mg kg^{-1} bw α-tocopherol respectively. Each group had a corresponding recovery group except the negative control. Drugs and vehicle administration was done orally for 8 weeks.

The animals were weighed before and after the treatment periods. The drug treated groups were sacrificed after 8 weeks of treatment while the recovery groups were sacrificed after an additional 4 weeks during which the drugs were withdrawn from the rats. The organs were removed and weighed. Blood was collected via cardiac puncture using a 5 mL syringe for testosterone assay. The left epididymis of each rat was immediately excised and semen was withdrawn from the caudal portion for sperm characteristics analysis.

Sperm characteristics analysis: Sperm characteristic analysis was performed as previously described (Raji *et al*., 2003, 2005, 2006a, b). Immediately after excision, the caudal epididymis was sliced open and the semen was squeezed on the pre-warmed microscope slide. Two drops of warm 2.9% sodium citrate were added to the semen. The slide was examined under the microscope using the X40 objective. The spermatozoa were seen to move in a wave-like fashion and different wave patterns ranging from an absence of waves (0%) to the appearance of prominent waves in very rapid motion (100%) were observed and scored for mass spermatozoal motility. Sperm viability was done using nigrosin/eosin stain. The stain is an isotonic mixture of 10% nigrosin and 4% eosin. Semen was squeezed on the microscope slide and 2 drops of the stain were added. A film of the mixture was smeared on a clean microscope slide and air-dried. The slide was examined under the microscope using the X40 objective. The live spermatozoa were seen as clear and the dead ones as pink-stained cells against a blue background. The numbers of stained and unstained sperms in the field were counted and the percentage viability was calculated. After assessing the sperm viability, the slide was observed under the microscope using X100 objective. A random observation of at least 200 spermatozoa was made and the average was taken for each form of sperm abnormalities. The right epididymis was placed in a tube containing 5 mL of normal saline and its volume was measured by displacement. The contents of the tube were then poured into a ceramic mortar in which the epididymis was macerated, to release the spermatozoa. The improved Neubauer haemocytometer was used to determine the sperm count. A clean cover slip was pressed firmly down on the slide and after thorough mixing a drop of the diluted semen sample was introduced under the cover slip by capillary action. The ruled area was located at the centre of the microscope field of view by means of the X10 objective and the cells were counted under the X40 objective.

Determination of superoxide dismutase (SOD): This was carried out using the RANSOD kit, with one modification. Instead of the whole blood sample, homogenized and filtered testis solution was used. The RANSOD kit contained mixed substrate (xanthine, 0.05 mmol L and I.N.T, 0.05 mmol L^{-1}, buffer (CAPS 40 mmol L^{-1}, pH10.2, EDTA, 0.94 mmol L^{-1}), xanthine oxidase standard (80 U L^{-1}), sample diluents (5.4 U L^{-1}) and phosphate buffer (0.01 mol L^{-1}, pH 7.0) (50 mL 0.2M KH_2PO_4 + 29.65 mL 0.2N NaOH made up to 1 L, with distilled water). The percentage inhibition of each sample was used to obtain the SOD units from a standard curve of the reconstituted and diluted RANSOD kit.

Testosterone assay procedure: This was carried out using the immunometrics direct human serum testosterone enzyme based immunoassay (EIA) kit. The assay was carried out in 5 steps as previously described (Raji *et al*., 2005, 2006). The EIA kit was obtained from Immunometrics (London, UK) and contained testosterone EIA enzyme

label, testosterone EIA substrate reagent and EIA quality control sample. A quality control was carried out at the beginning and the end of the assay to ascertain acceptability with respect to bias and within batch variation. The EIA kit used had a sensitivity level of approximately 0.3 nmo L^{-1} (0.1 g mL^{-1}) of testosterone. The intra- and inter-assay variations were 10.02 and 10.12 %, respectively.

Statistical analysis: Data were expressed as mean±SEM and analyzed using the students' t-test and ANOVA where applicable. $p<0.05$ was accepted as statistically significant.

RESULTS

Individual effect of metronidazole and tetracycline on body and organ weight: Both the control and the drug treated rats maintained a slight increase (about 10%) in body weight between the beginning of the study and autopsy (Table 1). There was no significant change in weight of the liver when compared with the control. The weight of the testis significantly ($p<0.05$) reduced in rats treated with metronidazole with or without α-tocopherol. The weight of the testis, epididymis and seminal vesicles also significantly ($p<0.05$) reduced in all tetracycline treated rats. Co-administration of tetracycline with α-tocopherol brought about a further significant ($p<0.01$) reduction in the weight of seminal vesicles and prostate gland. The weight of the prostate gland significantly ($p<0.05$) decreased in the metronidazole plus α-tocopherol treated rats. However, these changes were restored in recovery experiments (Table 1).

Individual effect of metronidazole and tetracycline on sperm parameters: Table 2 shows that metronidazole caused a significant ($p<0.05$) reduction in sperm count and motility. Co-administration of α-tocopherol with metronidazole or tetracycline caused further significant decrease ($p<0.01$) in sperm counts. However, the sperm concentration significantly returned ($p<0.05$) to pretreatment levels in recovery experiment. α-tocopherol did not produce any change in the number of abnormal spermatozoa in tetracycline groups, but when administered with metronidazole more abnormal spermatozoa were observed. The percentage of abnormal sperms recorded on co-administration of tetracycline with α- tocopherol was close to that obtained after 4 weeks recovery period (Table 2).

Individual effect of metronidazole and tetracycline on testicular superoxide dismutase (SOD) activity: Superoxide dismutase (SOD) activity increased in the testis of metronidazole and tetracycline with or without α-tocopherol treated rats. However the percentage increase in SOD activity was significantly lower in α-tocopherol-antibiotic treated rats (Table 3).

Individual effect of metronidazole and tetracycline on serum testosterone concentration: The results in Table 4 indicate that serum testosterone levels in

Table 1: Effect of metronidazole and tetracycline on body and organ weights in male albino rats

Treatments (n = 5)	Body weight (g)	Liver (g)	Testis (g)	Epididymis (g)	Seminal vesicle (g)	Prostate gland (g)
Control	240.83±0.8	3.56±0.23	0.63±0.03	0.23±0.01	0.57±0.04	0.14±0.00
α-tocopherol alone	224.20±2.0	3.09±0.22	0.47±0.03*	0.15±0.01*	0.12±0.02	0.13±0.02
Recovery	221.10±2.0	3.39±0.23	0.56±0.04	0.22±0.03	0.59 ±0.01	0.56±0.02
Metronidazole alone	220.00±0.00	3.87±0.11	0.58±0.02	0.18±0.01*	0.50±0.06	0.14 ±0.01
Recovery	234.17±8.2	3.48±0.19	0.64±0.02	0.20±0.00*	0.29±0.02*	0.14±0.01
Metronidazole+α-tocopherol	218.30±1.7	3.39±0.07	0.45±0.05*	0.15±0.01*	0.49 ±0.03	0.09±0.01*
Recovery	229.40±2.7	3.36±0.09	0.59±0.01	0.19±0.02	0.54±0.04	0.13±0.06
Tetracycline alone	230.00±3.65	3.82±0.02	0.55±0.02	0.15±0.00*	0.27±0.01*	0.12±0.00*
Recovery	240.00±0.00	3.68±0.14	0.48±0.02*	0.14±0.00	0.23±0.02*	0.14±0.01
Tetracycline+α tocopherol	215.00±3.41	3.72±0.12	0.47±0.02	0.15±0.00*	0.24±0.03*	0.08±0.01
Recovery	223.00±3.5	3.01±0.14	0.56±0.03	0.220±0.01	0.44±0.04	0.14±0.02

*p<0.05 with respect to negative control

Table 2: Effects of metronidazole and tetracycline on rat sperm motility and counts

Treatments (n = 5)	Percentage motility (%)	Sperm count (×106/mL)	Abnormal sperm morphology (%)
Control	97.17±0.65	15.94 ±0.73	9.11
α-tocopherol alone	78.33±1.67*	15.72±0.74	9.10
Recovery	91.23±0.59	16.03±1.20*	10.13
Metronidazole alone	60.83±0.83*	12.45±0.50*	23.06*
Recovery	77.25±0.81	16.94±0.64	19.13*
Metronidazole+α-tocopherol	58.00±0.77*	16.37±0.25	13.20
Recovery	65.04±0.79	15.50±0.54	14.10
Tetracycline alone	64.67±1.52	6.32±0.36*	29.80*
Recovery	76.00±0.00	16.28±2.60*	14.41
Tetracycline+α-tocopherol	55.00±0.00*	15.25±0.27	13.00
Recovery	67.01±0.04	15.60±0.31	11.00

*p<0.05 with respect to control

Table 3: Effect of metronidazole and tetracycline on superoxide dismutase (SOD) activity in the testes of male albino rats

Treatments (n = 5)	Testicular superoxide dismutase activity (SOD units mL^{-1})	Change in SOD with respect to group I (%)	Change in SOD with respect to group II (%)
Control	2.00±0.00	0.00	0.00
α-tocopherol alone	19.00±0.00	-5.00	0.00
Recovery	2.10±0.01	+5.00	+10.52
Metronidazole alone	95.33±1.52*	+375.00	+401.58
Recovery	7.83±0.65*	+291.50	+312.11
Metronidazole+α- tocopherol	6.00±0.00	+200.00	+215.79
Recovery	10.67±0.67*	+433.50	+461.58
Tetracycline alone	11.73±4.37*	+486.50	+517.37
Recovery	10.40±4.44*	+420.50	+447.37
Tetracycline + α- tocopherol	4.00±0.00	+100.00	+110.53
Recovery	6.33±0.33*	+216.50	+233.16

* = $p<0.05$; Calculated as: Test - Control ×100/Control

Table 4: Individual effect of metronidazole and tetracycline on serum testosterone concentration in albino rats

Treatment group (n= 5)	Semm testosterone concentration (nmol mL^{-1})	Change** in testosterone with respect to group I (%)	Change** in testosterone with respect to group II (%)
Control	4.37±0.04	0.00	0.00
α-tocopherol alone	1.30±0.00*	-70.25	0.00
Recovery	2.30±0.01	-47.34	+76.90
Metronidazole alone	1.30±0.00*	-70.25	0.00
Recovery	2.50±0.00*	-42.79	+92.20
Metronidazole+α-tocopherol	1.30±0.00*	-70.25	0.00
Recovery	2.80±0.22*	-35.93	+115.38
Tetracycline alone	1.30±0.00*	-70.25	0.00
Recovery	2.50±0.01*	-42.79	+92.31
Tetracycline+α-tocopherol	1.30±0.00*	-70.25	0.00
Recovery	2.60 ±0.00*	-40.50	+100.00

* = $p<0.05$; Calculated as: Test - Control ×100/Control

the metronidazole and tetracycline with or without α-tocopherol treated rats were significantly ($p<0.05$) lower than those of the control rats.

DISCUSSION

A review of literature suggests that antimicrobial agents can affect male reproductive functions including, sperm counts (Murdia *et al*., 1978), motility (Hargreaves *et al*., 1998), morphology (Toovey *et al*., 1981; Birnie *et al*., 1981), spermatogenesis (Timmermans, 1974) and germ cell integrity (Kushniruk, 1976). The precise mechanism(s) of action of antibiotics on male reproduction remain to be fully elucidated. Recently, Raji *et al*. (2006) reported the negative impact of ampicilllin and cloxacillin on male reproductive function of rats using *in vitro* and *in vivo* experimental models. In the present study, the role of α-tocopherol on the effects of an antiprotozoal-metronidazole and an antibiotic- tetracycline on male reproduction are presented. Alpha tocopherol is a powerful antioxidant with ability to scavenge Reactive Oxygen Species (ROS). It also prevents the oxidation of essential cellular components because it is an excellent lipid-soluble, chain-breaking antioxidant.

Free radicals have been proposed as a major cause of defective sperm function in cases of male infertility (Sharma and Agarwal, 1996). There is growing evidence that spermatozoa are protected from detrimental ROS effects by the powerful antioxidants in seminal plasma since disturbances of sperm functions by ROS have been demonstrated in the absence of seminal plasma (e.g., during epididymitis or after semen preparation). Tetracycline scavenge both O_2* and *OH and its free radical scavenging property may be partly related to its anti-ulcer effects (Suzuki *et al*., 1998). Using electro paramagnetic resonance spectroscopy, Bittner *et al*. (1999), detected gamma-irradiation induced free radicals within tetracycline-HCl-loaded microspheres. Tetracyclines are distributed to various body tissues, which include, human prostatic fluid and semen. Oxytetracycline has been used as a fluorescent stain, for condensed spermatid nuclei of the rat (Milch *et al*., 1958) and tetracycline-HCl is also a known fluorophor (Ericsson and Baker, 1967). Caudal epididymal spermatozoa from male rabbits treated with 25 mg kg^{-1} of tetracycline-HCl (intravenously) twice a day for two days did not fluoresce but these spermatozoa did fluoresce when placed with tetracycline *in vitro*. The seminal vesicles were found to fluoresce in the treated rabbits (Ericsson and Baker, 1967). Previous evidence had indicated that the attachment of tetracycline and spermatozoa was not detrimental to the motility, morphology or concentration of spermatozoa (Ericsson and Baker, 1967). However, Hargreaves *et al*. (1998) showed that tetracycline at concentrations as low as 2.5 μg mL^{-1} caused a significant dose-dependent inhibition in percentage rapid moving spermatozoa, mean

path velocity, straight line velocity and curvilinear velocity, but at 50 μg mL^{-1} tetracycline, all spermatozoa were static. The aforementioned information from the literature showed evidence of possible involvement of free radical/antioxidant mechanism in tetracycline effect on male reproductive functions especially sperm indices. To test this hypothesis, an assay was carried out to evaluate the superoxide dismutase (SOD) activity in the testis of the treated rats. In this study, administration of tetracycline with α-tocopherol did not lead to any significant change in sperm count, but it decreased sperm motility. Superoxide dismutase is a first line antioxidant in the body's defense against detrimental actions of free radicals and reactive oxygen species (Fridovich, 1986). An increase in superoxide dismutase activity will thus suggest increased generation and consequently, concentration of free radicals. Superoxide dismutase activity remarkably increased after tetracycline treatment and decreased progressively after treatment had been stopped. Co-administration of α-tocopherol with tetracycline prevented any significant increase in superoxide dismutase activity indicating involvement of free radical in the action of tetracycline.

The epididymis acts as a storage organ for mature spermatozoa. The increase in weight observed in the metronidazole and tetracycline treated rats could possibly be due to the reduced volume of contents in the epididymis. However, in the rats that were treated with α-tocopherol alone or in combination with metronidazole or tetracycline, a decrease in the epididymal weight was observed in spite of the increase in sperm counts. The tetracycline treated rats exhibited a higher percentage of abnormal sperm when compared with the control. There were more secondary abnormal spermatozoa indicating damage of the spermatozoa during storage in the epididymis. The weight of the seminal vesicle was also significantly lower in tetracycline treated rats and it remained lower even up to 4 weeks after tetracycline treatment had been stopped. A less pronounced but still significant weight loss was observed in the seminal vesicle and prostate gland when tetracycline was administered with α-tocopherol which seem to suggest that these effects were reversible and could possibly be ameliorated by co-administration of α-tocopherol. This assumption supports the observation that sperm motility, which is maintained by the secretions of the seminal vesicles and prostate glands (Ganong, 1999), only reduced slightly with the administration of α-tocopherol.

Metronidazole is known to have a radio sensitizing effect on tumour cells. The mechanism of its antibacterial action appears to be dependent on relative hypoxia in the target cells and may involve interaction with free radicals (Goldsmith, 1989). Metronidazole can also permeate all body tissues and fluids because of its small molecular weight. An increase in superoxide dismutase activity was also noticed in the metronidazole treated rats. This increase was prevented by co-administration with α-tocopherol. Metronidazole undergoes a one-electron reduction called the futile cycle (Re *et al*., 1997). The futile cycle produces nitro radical anions. The effect of metronidazole on sperm function may therefore be caused by an increase in these free radical anions. Co-administration of metronidazole with α-tocopherol led to a significant increase in sperm motility, while sperm counts remained similar to those of the control rats. This suggests that α-tocopherol could inhibit the negative actions of metronidazole on sperm function.

Testosterone and Follicle Stimulating Hormone (FSH) act on the seminal tubules to initiate and maintain spermatogenesis (Christensen, 1975). The reduced testosterone concentration on metronidazole or tetracycline administration might indicate that these drugs suppressed Leydig cell steroidogenesis. However, only slight increases were observed within 4 weeks of stoppage of treatment. These increases were still significantly lower than the control values indicating that a more prolonged time might be required for probable full restoration of normal level of these parameters. Testosterone levels were also low in the rats that received α-tocopherol, metronidazole or tetracycline despite the fact that α-tocopherol significantly increased the sperm count when administered alone and kept the counts relatively constant when administered with metronidazole or tetracycline. This makes the reduction in testosterone concentration mechanism of antireproductive action of these drugs controversial and therefore requires further studies.

In conclusion, the varied effects of metronidazole and tetracycline on some male reproductive functions could possibly be mediated via a reduction in testosterone level and in some cases probably via the free radical generating mechanism.

REFERENCES

Awobajo, F.O., Y. Raji, I.I. Olatunji-Bello, F.T Kunle-Alabi, A.O. Adesanya and T.O. Awobajo, 2006. Fourteen days oral administration of therapeutic dosage of some antibiotics reduced serum testosterone in male rats. Nig. J. Health Biomed. Sci., 591: 17-20.

Birnie, G.G., T.I.F. McLeod and G. Watkinson, 1981. Incidence of Sulphasalazine induced male infertility. Gut., 22: 452-455.

Bittner, B., K. Mader, C. Kroll, H.H. Borchert and T. Kissel, 1999. Tetracycline-HCl-loaded poly (DL-lactide-co-glycolide) microspheres prepared by a spray drying technique: Influence of gamma-irradiation on radical formation and polymer degradation. J. Controlled Release, 59: 23-32.

Cerny, A., 1996. Side effects and consequencies of frequently used antibiotics in clinical practice. Schweizerische Medizinische Wochenschrift. J. Suisse de Medizinische, 126: 528-534.

Christensen, A.K., 1975. Leydig Cells: In: Handbook of Physiology, Greep, P.O. and Astwood (Eds.), Washington DC. Am. Physiol. Soc. Section, 7: 57.

Ericsson, R.J. and V. F. Baker, 1967. Binding of tetracycline to mammalian spermatozoa. Nature, 214: 403-404.

Fridovich, I., 1986. Biological effects of the super oxide radical. Arch. Biochem. Biophys., 247: 1-11

Ganong, W.F., 1999. Energy balance, Metabolism and Nutrition. In: Review of Medical Physiology, 19th Edn., Appleton and Lange USA.

Giannattasio, A., M. Girotti, K. Williams, L. Hall and A. Bellastella, 1997. Puberty influences expression of phospholipid hydroperoxide glutathione peroxidase (GPX_4) in rat testis: Probable hypophysis regulation of the enzyme in male reproductive tract. J. Endocr. Invest., 20: 439-444.

Goldsmith, R.S., 1989. Antiprotozoal Drugs; In: Basic and Clinical Pharmacology. Katzung, B.G. (Ed.), 4th Edn., Prentice-Hall International Inc., pp: 645-665.

Hargreaves, C.A., S. Rogers, F. Hills, F. Rahman, R.J.S. Howell and S.T. Homa, 1998. Effects of co-trimoxazole, erythromycin, amoxycillin, tetracycline and chloroquine on sperm function *in vitro*. Hum. Reprod., 13: 1878-1886.

Kolettis, P.N., 2003. Evaluation of the subfertile man. Am. Fam. Physician, 68: 1265- 2172 .

Kushniruk, I.I., 1976. Effect of several antibacterial preparations on the concentration of nucleic acids in spermatogenic epithelial cells. Tsitol. Genet., 10: 342-344.

Milch, R.A., D.P. Rall, J.E. Tobie, 1958. Fluorescence of tetracycline antibiotics in bone. J. Bone Joint Surg., 40: 897-910.

Murdia, A., V. Mathur, L.K. Kothari and K.P. Singh, 1978. Sulphatrimethoprim combinations and male fertility. Lancet, 2: 375-376.

Nelson, C.M.K. and R.G. Bunge, 1974. Semen analysis: Evidence for changing parameters of male fertility potential. Fertil. Steril., 25: 503.

Punta, M.D., E.H. Charreau and O.P. Pignataro, 1996. Nitric oxide inhibits leydig cell steroidogenesis Endocrinology, 137: 5337-5343.

Raji, Y., U.S. Udoh, O.O. Mewoyeka, F.C. Ononye and A.F. Bolarinwa, 2003. Implication of reproductive endocrine malfunction in male antifertility efficacy of *Azadirachta indica* extract in rats Afr. J. Med. Sci., 32: 159-165.

Raji, Y., O.S. Akinsomisoye and T.M. Salman, 2005. Antispermatogenic activities of *M. lucida* extract in male albino rats. Asian J. Androl., 7: 405-410.

Raji, Y., A.K. Oloyo and A.O. Morakinyo, 2006a. Studies on the reproductive activities of methanol extract of *Ricinus communis* seed in male albino rats. Asian J. Androl., 8: 115-121.

Raji, Y., F.O. Awobajo Olufadekemi T. Kunle-Alabi, M.A. Gbadegesin and A.F. Bolarinwa, 2006b. *In vivo* and *in vitro* reproductive toxicity assessment of ampicillin and cloxacillin in mammalian models. Intl. J. Pharmcol., 2: 9-14.

Re, J.L., M.P. De Meo, M. Laget and H. Guiraud *et al*., 1997. Evaluation of the gonotoxic activity of metronidazole and dimetridazole in human lymphocytes by comet assay. Mut. Res., 375: 147-155.

Schlegel, P.N., T.S.K. Chang and F.F. Marshall, 1991. Antibiotics: Potential hazards to male infertility. Fertil. Steril., 55: 235-242.

Sharma, R.K. and A. Agarwal, 1996. Role of reactive oxygen species in male infertility. Urology, 48: 835-850.

Suzuki, Y.M.I.,T. Segami and M. Ito, 1998. Anti-ulcer effects of tetracycline in rats; antioxidants, quercetin, α-tocopherol, nifedipine and tetracycline. Jap., J. Pharmacol., 78: 435-441.

Timmermans, I., 1974. Influence of antibiotics on spermatogenesis. J. Urol., 112: 348-349.

Toovey, S., E. Hudson, W.F. Hendry and A.J. Levi, 1981. Sulfasalazine and male infertility: Reversibility and possible mechanism. Gut, 22: 445-451.