Antioxidant profile changes in reproductive tissues of rats treated with nicotine

I. P. Oyeyipo[1,2], Y. Raji[1], A. F. Bolarinwa[1]

[1]Department of Physiology, College of Health Sciences, Osun State University, Osogbo, Osun State, [2]Department of Physiology, Laboratory for Reproductive and Developmental Programming, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria

Address for correspondence:
Dr. I. P. Oyeyipo, Department of Physiology, College of Health Sciences, Osun State University, Osun State, Nigeria.
E-mail: greatibuks@yahoo.com

Received: 07.11.2013
Review completed: 14.01.2014
Accepted: 26.02.2014

Access this article online

Quick Response Code:

Website: www.jhrsonline.org

DOI: 10.4103/0974-1208.130823

ABSTRACT

OBJECTIVES: Nicotine intake has been associated with reduced fertility, although the mechanisms responsible are still unclear. However, oxidative stress has been repeatedly implicated as the leading cause of male infertility. This study was therefore designed to investigate the effects of nicotine administration on testicular oxidant and antioxidant system in male albino rats. **MATERIALS AND METHODS:** Forty male rats weighing between 150 and 180 g were divided into five groups and treated orally for 30 days. Group I, which served as the control received 0.2 ml/kg normal saline, Groups II and III received 0.5 mg/kg and 1.0 mg/kg body weight (BW) of nicotine respectively. The fourth and fifth groups were administered with 0.5 mg/kg and 1.0 mg/kg BW of nicotine, but were left untreated for another 30 days. Homogenate of testis and epididymis were assayed for lipid peroxidation and anti-oxidant enzyme. **RESULTS:** The results show a significant decrease ($P < 0.05$) in testicular glutathione peroxidase, glutathione reductase, catalase and superoxide dismutase while a significant increase ($P < 0.05$) was observed in testicular lipid peroxidation and nitric oxide level in both groups when compared with the control. **CONCLUSION:** This experiment established that nicotine administration is associated with decreased testicular antioxidant and increase testicular lipid peroxidation, which might be a mechanism by which nicotine induce infertility.

KEY WORDS: Antioxidant, lipid peroxidation, nicotine, rats, testes

INTRODUCTION

Nicotine intake through cigarette smoking has become a very common occurrence and a serious health and economic issue in most societies. It is documented that tobacco usage, according to the World Health Organization, is associated with approximately one-third of the world's population older than 15 years of age.[1]

Infertility which is a major health issue among couples of child-bearing age is also on the increase with approximately half of known causes of primary infertility attributed to male factor.[2] Studies have implicated nicotine as one of the main pharmacological substances responsible for inducing infertility due to cigarette smoking. These studies have shown that nicotine affects sperm function by depressing the sperm count motility, viability, normal morphology and reduce the weight of the testis.[3,4] Fertility studies also reveal a significant decrease in the libido of male rats treated with nicotine.[4] However, the mechanism by which nicotine causes male infertility is poorly understood.

Increased oxidative stress results from excess generation of reactive chemical species called free radicals from a number of sources and/or from decreased enzymic and non-enzymic antioxidant defenses.[5] Free radicals and other reactive species have been implicated in the progression of not <100 different diseases.[6] Thus, the interest in free radicals and oxidative stress has grown in recent times.

Elevated oxidative stress has been associated with an imbalance in the activity of the cardiac autonomic nervous system[7] and several other heart diseases.[8] Oxidative stress is also associated with high frequencies of single- and double-strand deoxyribonucleic acid (DNA) breaks[9] leading to decreased fertilization rates and poor embryo cleavage and quality since infertility cases have been associated with sperm samples containing a high frequency of damaged DNA.[10]

Nicotine has been documented to alter the oxidant and antioxidant balance in rat lymphocyte in a dose and time dependent manner[11] and alters lipid peroxidation and antioxidant enzyme in plasma and ovaries of female rats.[12] It is still uncertain if nicotine, the main pharmacological active substance in tobacco, is responsible for various deleterious effects due to free radical production because evidence has shown nicotine to have both antioxidant effects and pro-oxidant effects.[13] However, no study has investigated the effect of nicotine on male reproductive organs oxidant and antioxidant balance in rats despite the involvement of oxidative stress in male infertility.

The present study was therefore designed to investigate the effects of nicotine on oxidant and antioxidant balance in some reproductive organs of male albino rats during treatment and withdrawal periods.

MATERIALS AND METHODS

Treatments

Nicotine preparation

Nicotine hydrogen tartrate with product number 26,140 (95% nicotine) was purchased from BDH Chemical Ltd., Poole, England. The nicotine dosage prepared in normal saline for each group of animals was delivered at 0.5 mg/kg and 1.0 mg/kg body weight (BW). The working solutions were stored in foil-wrapped glass bottle at 4°C for no longer than 10 days.

The dosage used in this studies i.e. 0.5 mg/kg BW and 1.0 mg/kg BW was chosen in relation to human studies to be the amount of nicotine absorbed by taking 10 and 20 cigarettes respectively. This dosage was administered to mimic what obtains in tobacco smoking.[14]

Animal treatment

A total of 40 Wistar rats of comparable weights (150-180 g) were used for the study. They were housed in well-ventilated cages maintained at 25 ± 2°C, on a 12-h light-dark cycle. The rats were fed on standard rat chow and tap water without restriction. They were acclimatized for 2 weeks before the experimental period. Procedures involving animals and their care were performed in accordance with the guidelines of the Institution Animal Ethics Committee and the National Institutes of Health for the care and use of animals.

The rats were assigned randomly to one of the five experimental groups as follows with eight rats per group and treated orally using oral cannula. Group I, which served as the control and received 0.2 ml/kg normal saline, Groups II and III received 0.5 mg/kg and 1.0 mg/kg BW of nicotine respectively for 30 days. The fourth and fifth groups were administered with 0.5 mg/kg and 1.0 mg/kg BW of nicotine for 30 days, but were left untreated for another 30 days. The latter two groups served as recovery groups.

Organ collection

The animals were dissected and the organs of interest; testes and epididymis were removed, cleared of adherent tissues for lipid peroxidation and anti-oxidant assay.

Lipid peroxidation and anti-oxidant assay

Tissue malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP_x) and glutathione reductase (GSH-R) levels were assayed using standard laboratory methods described in previous studies.[15,16]

Determination of nitric oxide (NO) level

For the estimation of NO production, biochemical assessment of stable NO oxidative metabolites, nitrite and nitrate was performed. Assessment of nitrite and nitrate levels was based on the Griess method.[17] Data in this study presents the sum of nitrite and nitrate levels, which are NO metabolites.

Statistical analysis

Analysis of data was conducted with Statistical Package for Social Sciences Software (Version 13.0; SPSS Inc., IL, USA) for windows. Results were expressed as the mean ± SEM followed by pairwise comparison between test and control groups using Student's *t*-test. Differences between groups were considered to be significant at $P < 0.05$.

RESULTS

Effect of nicotine on mean testicular and epididymal GP_x

Rats treated with 0.5 mg/kg BW and 1.0 mg/kg BW showed a significant decrease ($P < 0.05$) in the mean testicular GP_x. However, an insignificant decrease ($P > 0.05$) was observed in the recovery group of treatment when values were compared with the control as shown in Figure 1.

The results showed that nicotine caused an insignificant decrease ($P < 0.05$) in the mean epididymal GP_x for both 0.5 mg/kg BW and 1.0 mg/kg BW treated group when compared with the control group. This trend was also recorded for the recovery group as shown in Figure 1.

Effect of nicotine on mean testicular and epididymal GSH-R

Oral administration of 0.5 mg/kg BW and 1.0 mg/kg BW of nicotine significantly decrease ($P < 0.05$) the mean testicular GSH-R when compared with the control counterpart. Although, this observation is dose dependent. Rats in the recovery groups of these treatment showed an insignificant decrease ($P > 0.05$) in their mean testicular reductase when compared with the control as shown in Figure 2.

The results showed an insignificant ($P > 0.05$) and a significant decrease ($P < 0.05$) in the mean epididymal GSH-R level for 0.5 mg/kg BW and 1.0 mg/kg BW respectively when compared with the control. However, the recovery group showed an insignificant decrease when values are compared with the control group as shown in Figure 2.

Effect of nicotine on mean testicular and epididymal SOD

Results showed that oral administration of 0.5 mg/kg BW nicotine and 1.0 mg/kg BW nicotine significantly decreased ($P < 0.05$) the mean testicular SOD when compared with the control group. Although, this observation is dose dependent. Rats in the recovery groups of these treatments showed an insignificant decrease in the mean testicular SOD when compared with the control as shown in Figure 3.

Oral administration of nicotine caused an insignificant and significant decrease ($P < 0.05$) in the mean epididymal SOD for 0.5 mg/kg BW and 1.0 mg/kg BW respectively. The recovery groups showed an insignificant decrease ($P > 0.05$) in the epididymal SOD when values were compared with the control group as shown in Figure 3.

Effect of nicotine on mean testicular and epididymal CAT

The mean testicular CAT level of rats treated with 0.5 mg/kg BW and 1.0 mg/kg BW was significantly decreased ($P < 0.05$) when compared with their control. This decrease was dose-dependent. There was an insignificant decrease ($P > 0.05$) in the mean testicular CAT level when compared with the control group as shown in Figure 4.

There was no significant ($P > 0.05$) change in the mean

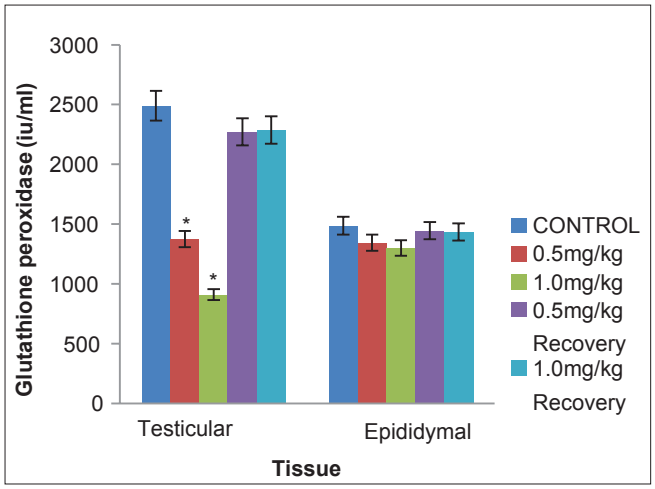

Figure 1: Tissue glutathione peroxidase level in male rats treated with nicotine. Values are expressed as mean ± SEM of 8 rats. *$P < 0.05$ versus control

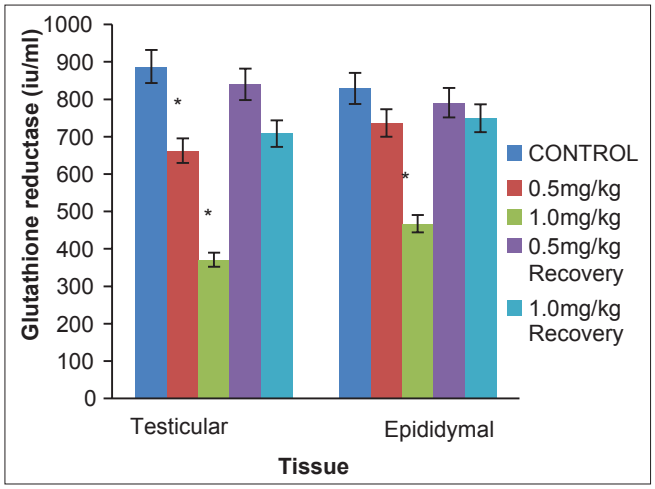

Figure 2: Tissue glutathione reductase level in male rats treated with nicotine. Values are expressed as mean ± SEM of 8 rats. *$P < 0.05$ versus control

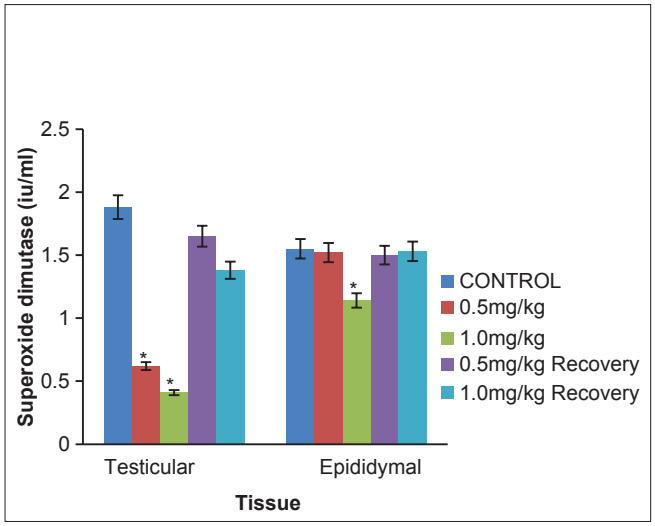

Figure 3: Tissue superoxide dismutase level in male rats treated with nicotine. Values are expressed as mean ± SEM of 8 rats. *$P < 0.05$ versus control

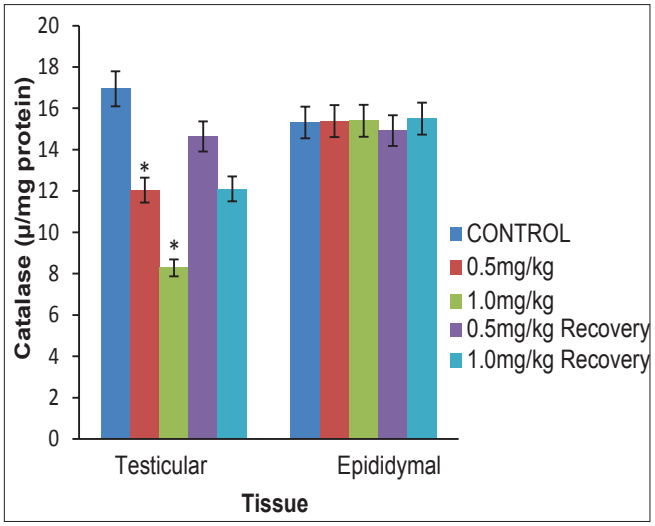

Figure 4: Tissue catalase level in male rats treated with nicotine. Values are expressed as mean ± SEM of 8 rats. *$P < 0.05$ versus control

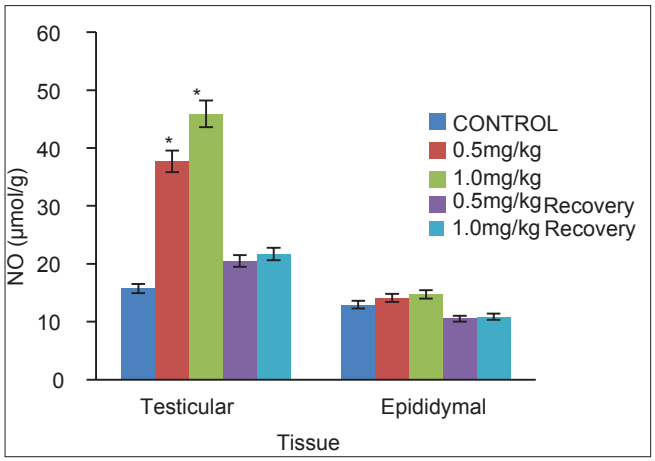

Figure 5: Tissue nitric oxide level in male rats treated with nicotine. Values are expressed as mean ± SEM of 8 rats. *P < 0.05 versus control

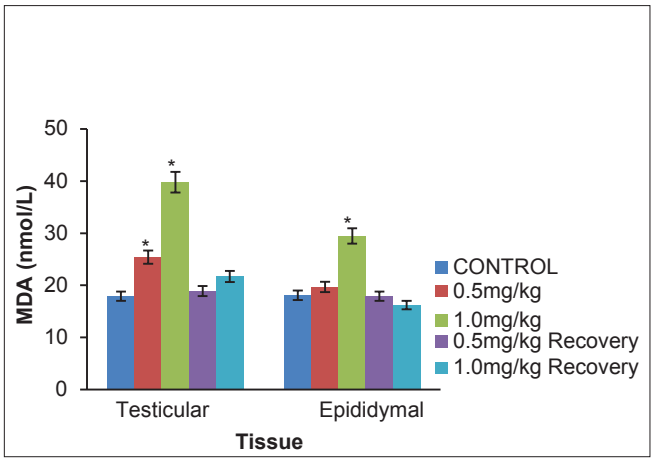

Figure 6: Tissue malondialdehyde level in male rats treated with nicotine. Values are expressed as mean ± SEM of 8 rats. *P < 0.05 versus control

epididymal CAT level in both treatment groups. The recovery groups also showed an insignificant decrease in the mean epididymal CAT level when values were compared with the control as shown in Figure 4.

Effect of nicotine on mean testicular and epididymal NO

The mean testicular NO level of rats treated with 0.5 mg/kg BW and 1.0 mg/kg BW was significantly increased (P < 0.05) when compared with their control. This decrease was dose-dependent. There was an insignificant increase (P > 0.05) in the mean testicular NO level when compared with the control group as shown in Figure 5.

There was no significant (P > 0.05) change in the mean epididymal NO level in both treatment groups. The recovery groups also showed an insignificant decrease in the mean epididymal NO level when values were compared with the control as shown in Figure 5.

Effect of nicotine on mean testicular and epididymal MDA

The mean testicular MDA level of rats treated with 0.5 mg/kg BW and 1.0 mg/kg BW was significantly increased (P < 0.05) when compared with their control. This increase was dose-dependent. There was an insignificant increase (P < 0.05) in the mean testicular MDA level of the recovery groups when compared with the control group as shown in Figure 6.

The results showed the 0.5 mg/kg BW group showed an insignificant increase (P > 0.05) while 1.0 mg/kg BW showed a significant increase (P < 0.05) in the mean epididymal MDA level when values were compared with the control. The recovery groups showed an insignificant decrease (P > 0.05) in the mean epididymal MDA level when values were compared with their respective treated groups as shown in Figure 6.

DISCUSSION

The present data showed that nicotine administration produced marked oxidative impact on the testis as evident by the significant increase in testicular lipid peroxidation and NO level as well as a significant decrease in antioxidants including GP_x CAT, SOD and GSH-R activities. This might suggest an inhibitory action of nicotine on enzymatic antioxidants in testes.

GP_x is reported to have a broad protective spectrum that metabolizes hydroperoxides. The suppression of the level of GP_x was observed in this study. However, the epididymal GP_x of rats was not significantly reduced, indicating that the effect of nicotine on the epididymis was not pronounced. Similarly, the recovery groups showed comparable values with the control indicating that the effect of nicotine on GP_x could be ameliorated by nicotine cessation.

In this study, nicotine inhibited GSH-R level in the testicular tissues *in vivo* compared with the control group. The epididymal GSH-R of rats was also significantly reduced only in the high treated group, however, the recovery groups showed comparable values with the control indicating that this effect of nicotine on GSH-R could be ameliorated by nicotine cessation.

The decrease in testicular GP_x levels in nicotine-treated rats might also be in part, attributed to the inhibition of GSH-R levels, this is responsible for regeneration of GP_x from its oxidized form.

This study also suggests that the activity of SOD which catalyzes the dismutation of superoxide (O_2^-) radical to hydrogen peroxide (H_2O_2)[18] was down-regulated in the testis in both the low and high treated group, although these effects were dose dependent. Epididymal SOD was reduced

only in the high treated group indicating that low level of nicotine had no significant effect on epididymal activities of SOD. The recovery groups showed similar values with the control indicating that the effect of nicotine on SOD could be ameliorated by nicotine withdrawal. The observed decrease in the testicular SOD level may be a consequence of decreased *de novo* synthesis of enzyme proteins or oxidative inactivation of enzyme protein.

The inhibition of testicular SOD activity might also be attributed to either hyperglycemia as reported by Sharpe *et al*. who found that glucose induced oxidative stress in different tissues and/or due to loss of enzyme cofactors namely copper and zinc.[19] In addition, previous studies have also indicated an increase in glucose level with nicotine administration.[20] Administration of nicotine significantly decreased CAT activity in the testes. The primary role of CAT is to scavenge H_2O_2 that has been generated by free radicals.[21] The recovery groups showed similar values with the control indicating that the effect of nicotine on CAT could be ameliorated by nicotine withdrawal.

This study also demonstrated that nicotine administration is associate with an increase NO level in the testes. This observation is in consonance with the previous study.[22]

Nicotine treated rats showed an elevation in MDA level when compared with the control group. An increased MDA concentration might be a consequence of decreased production of antioxidants in the nicotine treated rats' tissues thereby shifting the delicate balance in favor of ROS thus leading to a plethora of pathologic damage to sperm cells and concomitant loss of function. Lipid peroxidation of unsaturated fatty acids is a frequently used indicator of increased oxidative stress and subsequent oxidative damage.[11] The reduced level of SOD and CAT activity might generate excessive H_2O_2, which could give rise to other ROS such as hydroxyl radicals.[6] This finding corresponds with earlier studies when the rats were exposed to cigarette smoke for 45[23] and 60 days.[24] The effects of nicotine appear to be significant in the testis, but not so in the epididymis in this study. This might be that the tissues of the testis are more susceptible and vulnerable to oxidative stress and damage than the epididymis. This study further shows that down-regulation of antioxidant enzyme levels in the treated rats is a mechanism by which nicotine induces infertility in male. This study also confirmed that oxidative stress might be a mechanism by which nicotine causes infertility in male rats.

REFERENCES

1. Mohammed AG, Mohammed A, Behrooz M. *in vitro* inhibition of human sperm creatinine kinase by nicotine, cotinine and cadmium as a mechanism in smoker men infertility. Int J Fertil Steril 2008;2:125-30.
2. Yeşilli C, Mungan G, Seçkiner I, Akduman B, Açikgöz S, Altan K, *et al*. Effect of varicocelectomy on sperm creatine kinase, HspA2 chaperone protein (creatine kinase-M type), LDH, LDH-X, and lipid peroxidation product levels in infertile men with varicocele. Urology 2005;66:610-5.
3. Oyeyipo IP, Raji Y, Emikpe BO, Bolarinwa AF. Effects of oral administration of nicotine on organ weight, serum testosterone level and testicular histology in adult male rats. Niger J Physiol Sci 2010;25:81-6.
4. Oyeyipo IP, Raji Y, Emikpe BO, Bolarinwa AF. Effects of nicotine on sperm characteristics and fertility profile in adult male rats: A possible role of cessation. J Reprod Infertil 2011;12:201-7.
5. Agarwal A, Nallella KP, Allamaneni SS, Said TM. Role of antioxidants in treatment of male infertility: An overview of the literature. Reprod Biomed Online 2004;8:616-27.
6. Halliwell B, Gutteridge JM. Free Radicals in Medicine and Biology. Oxford: Oxford University Press; 1999.
7. Manzella D, Barbieri M, Ragno E, Paolisso G. Chronic administration of pharmacologic doses of vitamin E improves the cardiac autonomic nervous system in patients with type 2 diabetes. Am J Clin Nutr 2001;73:1052-7.
8. Dhalla NS, Liu X, Panagia V, Takeda N. Subcellular remodeling and heart dysfunction in chronic diabetes. Cardiovasc Res 1998;40:239-47.
9. Aitken RJ, Koopman P, Lewis SE. Seeds of concern. Nature 2004;432:48-52.
10. Sakkas D, Urner F, Bizzaro D, Manicardi G, Bianchi PG, Shoukir Y, *et al*. Sperm nuclear DNA damage and altered chromatin structure: Effect on fertilization and embryo development. Hum Reprod 1998;13 Suppl 4:11-9.
11. Das S, Chakraborty SP, Roy S, Roy S. Nicotine induced pro-oxidant and antioxidant imbalance in rat lymphocytes: *In vivo* dose and time dependent approaches. Toxicol Mech Methods 2012;22:711-20.
12. Chattopadhyay K, Chattopadhyay BD. Effect of nicotine on lipid profile, peroxidation and antioxidant enzymes in female rats with restricted dietary protein. Indian J Med Res 2008;127:571-6.
13. Newman MB, Arendash GW, Shytle RD, Bickford PC, Tighe T, Sanberg PR. Nicotine's oxidative and antioxidant properties in CNS. Life Sci 2002;71:2807-20.
14. Becker RF, Little CR, King JE. Experimental studies on nicotine absorption in rats during pregnancy. 3. Effect of subcutaneous injection of small chronic doses upon mother, fetus, and neonate. Am J Obstet Gynecol 1968;100:957-68.
15. Adewole SO, Ojewole JA. Protective effects of *Annona muricata* Linn. (Annonaceae) leaf aqueous extract on serum lipid profiles and oxidative stress in hepatocytes of streptozotocin-treated diabetic rats. Afr J Tradit Complement Altern Med 2008;6:30-41.
16. Khatibi F, Yaghoubi AR, Rahbani NM. Study of antioxidant enzymes, lipid peroxidation, lipid profile and immunologic factor in coronary artery disease in East Azarbijan. Int J Med Biomed Res 2012;1:147-52.
17. Cortas NK, Wakid NW. Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. Clin Chem 1990;36:1440-3.
18. McCord JM, Keele BB Jr, Fridovich I. An enzyme-based theory of obligate anaerobiosis: The physiological function of superoxide dismutase. Proc Natl Acad Sci U S A 1971;68:1024-7.
19. Sharpe PC, Liu WH, Yue KK, McMaster D, Catherwood MA, McGinty AM, *et al*. Glucose-induced oxidative stress in vascular contractile cells: Comparison of aortic smooth muscle cells and retinal pericytes. Diabetes 1998;47:801-9.
20. Alada AR. Effects of calcium channel blockers on nicotine-induced hyperglycemia in the rat. Afr J Med Med Sci 2001;30:57-9.
21. Ribière C, Hininger I, Rouach H, Nordmann R. Effects of chronic ethanol

administration on free radical defence in rat myocardium. Biochem Pharmacol 1992;44:1495-500.
22. Swami S, Suryakar AN, Katkam RV, Kumbar KM. Absorption of nicotine induces oxidative stress among bidi workers. Indian J Public Health 2006;50:231-5.
23. Rajpurkar A, Dhabuwala CB, Jiang Y, Li H. Chronic cigarette smoking induces an oxidantantioxidant imbalance in the testis. J Environ Pathol Toxicol Oncol 2000;19:369-73.
24. Ozyurt H, Pekmez H, Parlaktas BS, Kus I, Ozyurt B, Sarsilmaz M. Oxidative stress in testicular tissues of rats exposed to cigarette smoke and protective effects of caffeic acid phenethyl ester. Asian J Androl 2006;8:189-93.

How to cite this article: Oyeyipo IP, Raji Y, Bolarinwa AF. Antioxidant profile changes in reproductive tissues of rats treated with nicotine. J Hum Reprod Sci 2014;7:41-6.
Source of Support: The authors acknowledge the Tertiary Education Trust fund (TETfund) of Nigeria for providing fund for this research work,
Conflict of Interest: None declared.

Author Help: Online submission of the manuscripts

Articles can be submitted online from http://www.journalonweb.com. For online submission, the articles should be prepared in two files (first page file and article file). Images should be submitted separately.

1) **First Page File:**
Prepare the title page, covering letter, acknowledgement etc. using a word processor program. All information related to your identity should be included here. Use text/rtf/doc/pdf files. Do not zip the files.
2) **Article File:**
The main text of the article, beginning with the Abstract to References (including tables) should be in this file. Do not include any information (such as acknowledgement, your names in page headers etc.) in this file. Use text/rtf/doc/pdf files. Do not zip the files. Limit the file size to 1 MB. Do not incorporate images in the file. If file size is large, graphs can be submitted separately as images, without their being incorporated in the article file. This will reduce the size of the file.
3) **Images:**
Submit good quality color images. Each image should be less than 4096 kb (4 MB) in size. The size of the image can be reduced by decreasing the actual height and width of the images (keep up to about 6 inches and up to about 1800 x 1200 pixels). JPEG is the most suitable file format. The image quality should be good enough to judge the scientific value of the image. For the purpose of printing, always retain a good quality, high resolution image. This high resolution image should be sent to the editorial office at the time of sending a revised article.
4) **Legends:**
Legends for the figures/images should be included at the end of the article file.