Effect of Nicotine on the Testis of Adult Albino Rat and the Possible Protective Effect of Vitamin E.

Mohamed Abdel Haye Autifi M.D, Alaa Sayed Elsagheir M.D and *Mohammed* AbdeLataif Almandouh

Human Anatomy and Embryology Department, Faculty of Medicine, AL-Azhar University, Egypt.
dr_abo7afsa32@yahoo.com

Abstract: Introduction: The harmful effects of nicotine on male fertility have been reported in experimental and clinical studies. However, the protective effect of vitamin E against this toxicity and the reversibility of nicotine induced testicular toxicity after its withdrawal is still a matter of controversy. **Aim of the Work:** to examine the effect of nicotine administration on albino rat testis and to study the potential role of nicotine withdrawal and vitamin E on the amelioration of the nicotine effect on the testis. **Materials and Methods:** forty male albino rats were maintained for 30 days as follows: Group 1, control group, group 2, nicotine group [1mg/kg/day, intrapertonial (IP)], Group 3, nicotine withdrawal [1mg/kg/day, intrapertonial (IP)] then scarified 30 days after the last dose, group 4, nicotine + vitamin E [100mg/kg/day (IP)]. At the end of experimental study, testicular tissues and blood samples were taken for histological and laboratory studies. **Results:** Comparing with the control it was found that, simultaneous administration of vit. E with nicotine and nicotine withdrawal show edprotective effect against Histotoxicity and laboratory results of nicotine, through increase in the thickness of germinal epithelium with significant increase in the size of the seminiferous tubules, spermcount& significant increase in serum testosterone level. **Conclusion:** The present results seem to be rather surprising in view of the fact that nicotine could destroy testicular tissues in short time duration while nicotine withdrawal and vit. E could produce partial protection. Further studies are necessary to illuminate the other dark sides of nicotine on infertility in human and to discover others protective agents against its toxicity.

[Mohamed Abdel Haye Autifi, Alaa Sayed Elsagheir and Mohammed AbdeLataif Almandouh. **Effect of Nicotine on the Testis of Adult Albino Rat and the Possible Protective Effect of Vitamin E.** *Nat Sci* 2017;15(4):13-19]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 2. doi:10.7537/marsnsj150417.02.

Key Words: Nicotine, Testes, α-tocopherol, Fertility.

1. Introduction

Cigarette smoking is a highly addictive personal habit among teenager and young adults ***(1)***. Tobacco smoke is a mixture of more than 4000 components including carcinogens, oxidants and aldehydes, all of which have the potential to cause inflammation and damage of cells ***(2).*** One of the most abundant organic particles in cigarette smoke is nicotine, which is a very toxic alkaloid responsible for some positive and negative effect on the various organs. Smoking has been associated with carcinogenesis, cardiovascular diseases and other organ disorders ***(3).*** It is well documented that there is a significant association between smoking and reduced fertility among male smokers ***(4).*** Nicotine administration induces changes in gonadal function and deficiency in sperm maturation and spermatogenesis and has detrimental effect on the sperm fertilizing potential of male rats ***(5).*** In addition to its toxic effects on gonadal function in male, it also lowers testosterone level in serum ***(6).*** Natural antioxidants as polyphenols of vitamin E have received much attention for the treatment of oxidative-stress-related pathological conditions ***(7)***. Vitamin E is a natural antioxidant its ameliorating effect on genital organs was reported by ***(8)***.

2. Material and Methods

Animal Used:

Forty adult male wistar albino rats of 8-10 weeks and weighting 200-250 gm were used. The animals were obtained from animal house, Faculty of Medicine, Cairo University May 2016. They were housed in spacious wire mesh cages in a well-ventilated room and were kept under a constant day/night cycle in a climate controlled condition with an access to food and water. All experiments were carried out according to the guidelines of the Institutional Animal Ethics Committee.

Chemicals:

The chemicals in this experiment were nicotine ((S)-3-(1- Methyl-2-Pyrroli-dinyl) pyridine) and vitamin E. Nicotine was supplied as a white powder containing 100 mg nicotine which was dissolved in 100 ml saline so each 1 ml contain 1mg nicotine, purchased from SIGMA-ALDRICH. Vitamin E extract was supplied as a 100 ml solution containing 1 gm vitamin E so each 10 ml contain 100 mg vitamin E. vitamin E was obtained from Faculty of Pharmacy, AL-Azhar University, Egypt.

Experimental Design:

After two weeks of acclimatization, the rats were randomly divided into control and experimental

groups:

Group 1:

Formed of 10 rats. Injected with 1 ml 0.9 % sodium chloride (Nacl) intraperitoneal (IP) daily throughout the duration of the study.

Group 2 (nicotine exposure):

Formed of 10 rats injected with nicotine (1 mg/kg body weight) (IP) every day for 28 days and scarified at day 29 ***(9).***

Group 3(nicotine withdrawal):

Formed of 10 rats injected (IP) with nicotine(1 mg/kg body weight)every day for 28 days andscarified 30days after the last dose***(9).***

Group 4(nicotine and vitamin E treatment):

Formed of 10 rats injected IP with nicotine **(**1 mg/kg body weight) and vitamin E (100 mg/kg body weight) every day for 28 and scarified at day 29 ***(9)***.

Specimens collection:

At the assigned times, the rats were anaesthetized using ether inhalation, and blood samples were obtained by direct left ventricle puncture for serum testosterone measurement. Then the testes were dissected out, preserved in 10% buffered formalin and then processed for paraffin sections.

Processing of the specimens for light microscopic examination:

The specimens were processed for paraffin sections by gradual dehydration using ascending graded concentrations of alcohol, cleared in xylene and embedded in soft and then in hard paraffin wax.

Transverse sections were cut at 5-6 µm and treated as follows:

1. Some sectionswere stained with Hematoxylin and Eosin (H&E) forevaluation of histopathological changes.
2. Other sections were stained with AgNor for detection of mitotic activity.

Testosterone level assessment:

At the end of the experiment serum testosterone was measured in Yomna laboratory AlmahalaAlkobra EGYPT for all groups by VIDAS Testosterone. It is an automated quantitative test for use on the instruments of VIDAS family for the enzyme immunoassay measure of total testosterone in serum or plasma, using the ELFA technique (Enzyme Linked Fluorescent Assay). It is intended in the diagnosis and management of conditions involving excess or deficiency of this androgen.

Quantitative analysis of cell proliferation rate (AgNOR proteins)

Images were captured using an Olympus LC20 digital camera (Japan) coupled to a binocular Olympus microscope (Japan) with 1000X magnification. The images were captured for each group for 30 consecutive microscopic fields. The number of AgNOR dots per nucleus in the spermatocytes was quantified by visual count ***(10)***. The AgNOR count per nucleus was calculated for each specimen.

3. Results:

1. Histological results

Group 1: (Hematoxylin& Eosin)

The testicular tissue of adult albino rat of control group showed circular seminiferous tubules separated by interstitial tissues. The seminiferous tubules were lined by 5-9 rows of stratified epithelium composed of two categories of cells, spermatogenic cells and Sertoli cells resting on a clear basement membrane. The spermatogenic cells showed various stages of spermatogenesis and spermiogenesis. The primary spermatocytes were noticed as large cells located closer to the lumen of the tubule than the spermatogonia. Their nuclei were round or oval with one or more nucleoli. The chromatin is finely dispersed inside the nucleus. The spermatids were arranged in several rows. They have spherical or oval neuclei. Spermatozoa were present free in the lumen of seminiferous tubules. Sertoli cells were pyrimidal cell resting up on the basement membrane, they have large ovoid vesicular nuclei, while cytoplosmic outlines cannot be seen distinctly. The interstitial tissue was lying in between the seminiferous tubules and containing blood vessels, bundles of collagen fibers and Leydig cells. They were polyhedral with finely-dispersed chromatin and oval or spherical nuclei (fig.1).

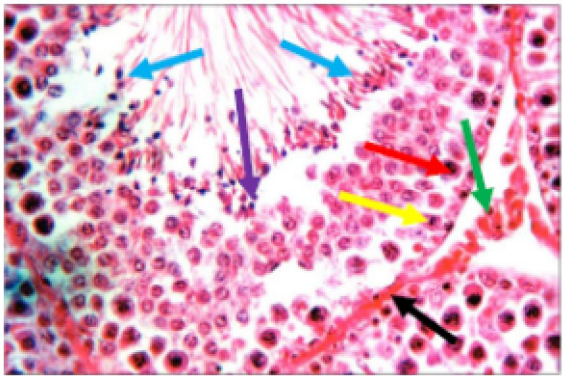

Fig.1: A photomicrograph of a section of the testis of control adult albino rat showing average sized S.T and average B.M (black arrow), Sertoli cell (yellow arrow), average germinal linning with 1ry spermatocyte appeared as large rounded cell above the spermatogina (red arrow), spermatid (violet arrow) and many spermatozoa (blue arrow) and average interstitium containing leydig cells (green arrow).
(H & E; X400)

Group 1: (AgNOR).

AgNOR dots were located strictly within the nuclei of the spermatocytes.

They were clearly visible as black dots in control group (fig.2).

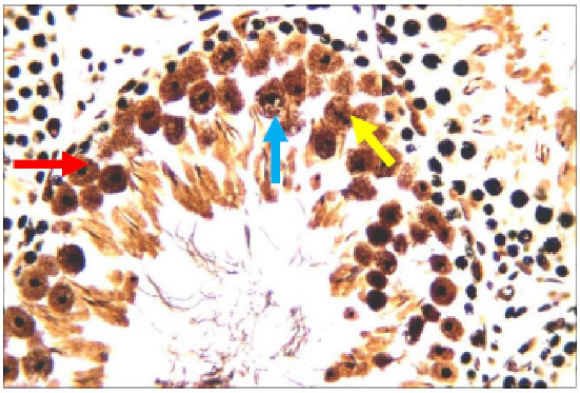

Fig.2: A photomicrograph of section of the testis of control adult albino rat showing one AgNOR dot / nucleus (red arrow), 4 dots (yellow arrow) and 5 dots (blue arrow). (AgNor stain X400)

Group 2: (Hematoxylin & Eosin)

The testicular tissue of adult albino rat nicotine treated group showed shrinkage of seminiferous tubules and wide interstitial spaces between seminiferous tubules. Stratified epithelium lining semineferous tubules was formed of two or three raws of spormatogenic cells including Sertoli cells denoting arrest of spermatogenesis.

Some seminiferous tubules appeared small in diameter, distorted and degenerated with thickened BM while other S.T showing incomplete spermatogenesis and empty lumen but some seminiferous tubules appeared intact with complete spermatogenesis. All these S.T were widely separated with distorted interstitial tissue containing dilated blood vessels, few irregular spindle shaped leydig cells. Spermatogonia in addition to primary spermatocytes could be identified inside the seminiferous tubules. The germinal lining was necrotic with few spermatids no sperms and disrupted basement membrane.

Sertoli cells were lying up on the basement membrane, they appeared more or less triangular with bases lying on the basement membrane while apices directed towards the.

The primary spermatocytes were found hardly inside the seminiferous tubules, some of them had ruptured nucleus with consequence scattering of their chromatin content. No spermatids or spermatozoa in most of the seminiferous tubules. (fig.3).

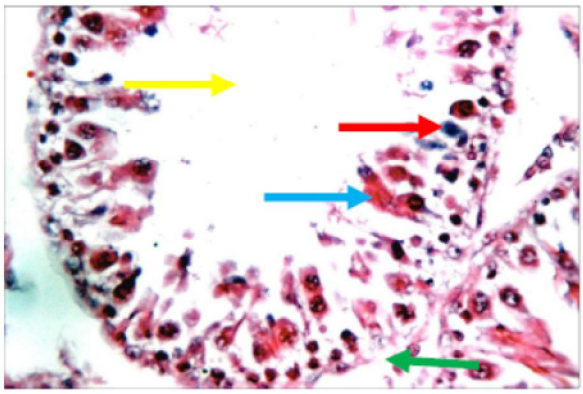

Fig.3: A photomicrograph of a section of the testis of adult albino rat treated with nicotine for one month showing seminiferous tubule with Sertoli cells (red arrow), necrotic germinal lining (blue arrow), no sperms (yellow arrow) and disrupted BM (green arrow). (H & Eosin; X360).

Group 2: (AgNOR).

The least number of AgNOR dots was present in nicotine treated group indicating mitotic arrest and decreased cell proliferation. (fig.4).

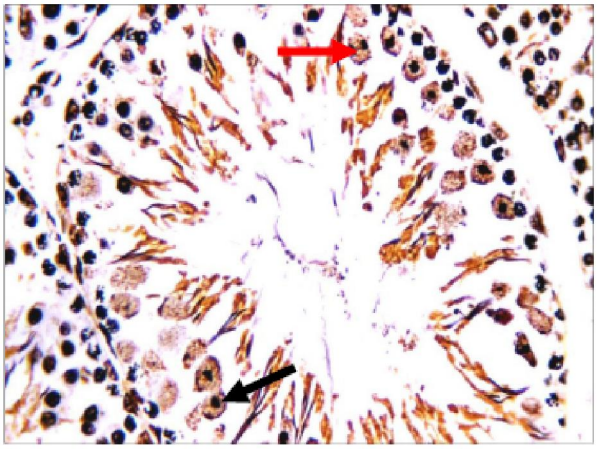

Fig.4: A photomicrograph of section of the testis of adult albino rat treated with nicotine for one month showing few AgNor dots, one dot (black arrow) and two dots (red arrow). (AgNor stain x 400)

Group 3: (Hematoxylin& Eosin)

Most of the tubules were average in size with average B.M with complete spermatogenesis showing spermatogonia, primary spermatocytes, spermatids and mature sperms. These healthy tubules were separated by average interstitium increased number of polygonal Leydig cells but few S.T appeared necrotic, degenerated with no sperms. Seminiferous tubules were returning back to normal showing complete spermatogenesis with thin germinal lining, scattered spermatids and spermatozoa with average interstitium

showing increased number of polygonal Leydig cells (fig. 5).

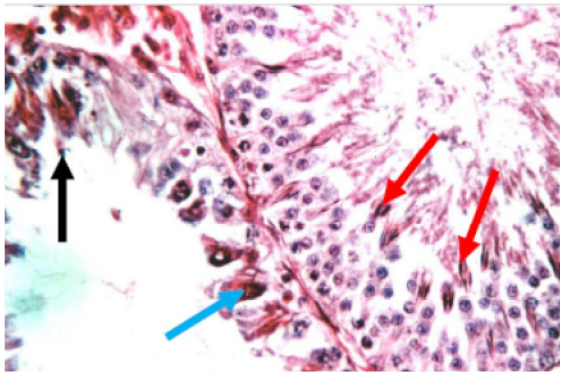

Fig 5: A photomicrograph of a section of the testis of adult albino rat treated with nicotine for one month and scarified one month after the last dose showing one seminiferous tubule with complete spermatogenesis (red arrow) and another one showing scattred necrotic germinal cells (blue arrow) with few spermatids (black arrow). (H & Eosin; X360)

Group 3: (AgNOR). AgNOR dots were visible as black dots in group 3 with increasing dots indicating mitotic activity and cellular proliferation (fig 6)/

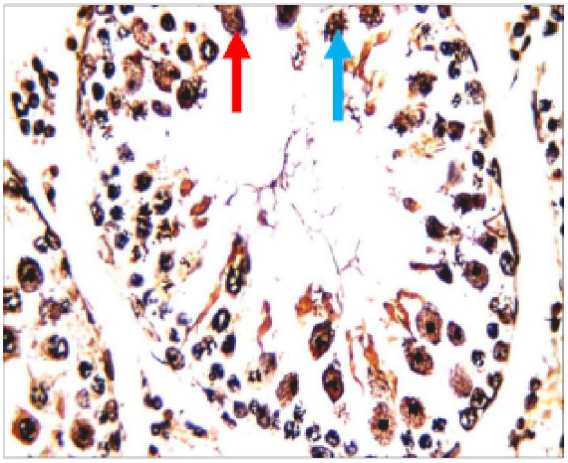

Fig. 6: A photomicrograph of section of the testis of adult albino rat of withdrawal group showing numerous irregularly shaped AgNOR dots 5 dots (red arrow) and 7 dots (blue arrow). (AgNor stain X400)

Group 4: (Hematoxylin& Eosin)

The seminiferous tubules started to return back to normal showing circular S.T which was average size with average B.M, complete spermatogenesis in the form of dark spermatogonia, primary spermatocytes, spermatids and mature sperms. Sertoli cells were present separating the columns of germinal cells. The seminiferous tubules also separated by average interstitil tissue which contains polygonal leydig cells.(fig. 7).

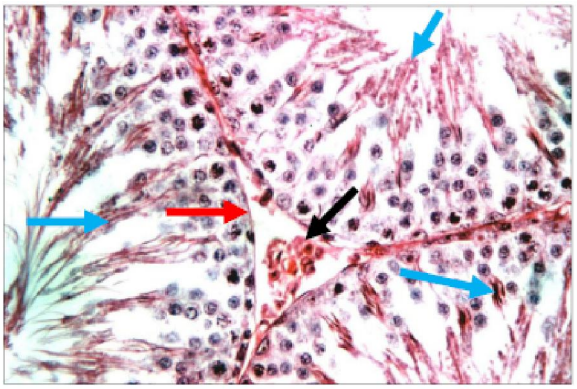

Fig. 7: A photomicrograph of a section in the testis of adult albino rat treated with nicotine and vitamin E for one month showing average sized seminiferous tubules with complete spermatogenesis up to spermatozoa (blue arrow) with average B.M (red arrow) and prominantinterstitil tissue containing polygonal Leydig cells (black arrow). (H & Eosin; X360).

Group 4: (AgNOR).

AgNOR dots were clearly visible as black dots in nicotine and vitamin E treated with variable number more than that of nicotine treated group indicating better cellular proliferation.(fig. 8)

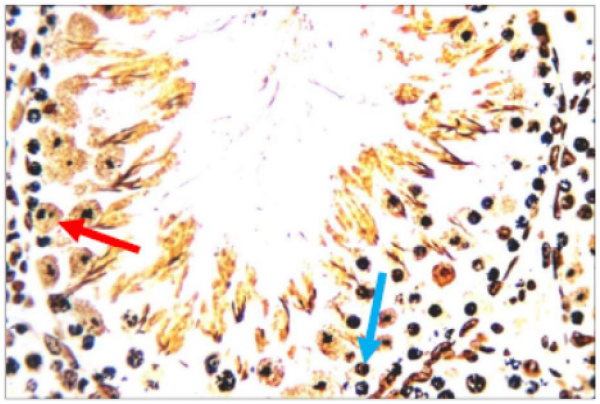

Fig. 8: A photomicrograph of section of the testis of adult albino rat treated with nicotine and vitamin E for one month showing numerous irregularly shaped AgNOR dots 2 dots (red arrow) and 5 dots (blue arrow). (AgNor stain X400)

Laboratory results:

There was highly significant decrease in testosterone hormone obtained from nicotine treated group for one month (11.4 ng/ml) as compared to that of control group (12.89 ng/ml). On the other hand, there was insignificant decrease in testosterone

hormone in nicotine and vitamin E treated group for one month (12.70 ng/ml) and withdrawal group (12.55 ng/ml).

Table 1: Testosterone level measurements of all groups.

Group	Testosterone (ng/ml)
Control	12.89
Nicotine	11.41
Nicotine withdrawal	12.55
Nicotine + Vitamin E	12.70

4. Discussion

Nicotine administration induces changes in gonadal function, deficiency in sperm maturation, spermatogenesis and has detrimental effect on the sperm fertilizing potential of male rat**.** In addition to its toxic effects on gonadal function in male, it also lowers testosterone level in serum. The present work aimed to study the more closely effect of this widely used substance either under medical supervision or not in order to put the bases for the best way of use of this drug either to avoid many hazards that may develop due to uncontrolled use of this substance. The present study has been demonstrated due to new trends in nicotine uses as nicotine replacement therapy, preeclampsia, atopic disorders such as allergic asthma and frontal lobe epilepsy to study its possible toxic effect on the testis. The adult albino rat was used in this study due to its similarity to testicular architecture of human, the onset of puberty is around the age of 7-9 weeks and when the average weight is about 200 gm***(11)***. Vitamin E was used due to its ability to reduce oxidative stress induced by toxic substances such as nicotine. In the present study the specimens were stained by Haematoxylin and Eosin and AgNOR stain for histological study. Haematoxylin and Eosin stain is the best stain that can demonstrate the acidophilic and basophilic components of the cell, and AgNOR was used to demonstrate mitotic activity and cellular proliferation. The testes of the adult treated rats were reduced in size and the seminiferous tubules appeared shrunken and separated by wide interstitial spaces. Some of the seminiferous tubules were highly affected showing collapse or degeneration of spermatogenic cells with consequence arrest of spermatogenic process. In the collapsed cases the seminiferous tubules were characterized by absence of spermatozoa in the center. The less affected tubules showed thickening of their basement membrane with some degenerated and abnormal cells with low number of spermatozoa in the center of tubules. The other tubules however appeared with normal architecture, and the spermatogenic and Sertoli cells appeared more or less normal in these tubules. The interstitial cells of Leydig appeared few in number and atrophied if compared with the control group. The primary spermatocytes the control group. The primary spermatocytes of the treated group showed marked nuclear changes where its nuclear membrane is ill-defined and disrupted. The primary spermatocytes were the most affected cells after the spermatids and spermatozeo appeared more or less deformed. AgNOR reactive dots could be demonstrated in the nuclei of nicotine treated animals showed much decreased number when compared with control group, which indicates arrest of their mitotic activity. In the present study, the testes of the treated group showed shrunken or collapsed seminiferous tubules. These results are in agreement with***(12) and (13).*** They stated that treatment with nicotine was associated with atrophy of the testis and suppression of spermatogenesis in rats and attributed these changes to gonadotrophins inhibition. Also, our results were in agreement with the results obtained by ***(14)***who observed a decrease in seminiferous tubule diameter in rats exposed to nicotine which can be considered as the cause of the reported decrease testosterone level. In agreement with ***(9),*** the present study showed that some of the seminiferous tubules were highly affected showing collapse or degeneration of spermatogenic cells leading to arrest of spermatogenic process. This is in disagreement with ***(5)*** who reported significant gradual increase in the number of spermatogonia with decrease in the number of spermatocytes and spermatids indicating slow conversion of spermatogonia to spermatocytes and spermatids, our study indicates that the number of spermatogonia also decreased. In agreement with, ***(15),*** who stated that the histological section showed testicular degeneration and disorganization in the cytoarchitecture. In the collapsed cases the seminiferous tubules were characterized by absence of spermatozoa in the center. I agree with ***(9)*** who reported that nicotine induced significant reduction in sperm count, sperm motility, sperm viability and normal sperm cells. The basement membrane of all seminiferous tubules of the present study appeared thickened. Our results were in agreement with ***(16).*** They reported similar results and explained these changes due to the increased number of the abnormal and degenerated cells in man and rat testes as a result of systemic toxicity of the drug. Another suggestion by the same authors was that alteration in the basement membrane structure and / or function could lead to blockage of free flow of nutrients across the barrier, thus adversely affecting the germinal cells. The interstitial cells of Leydig appeared few in number and atrophied if compared with the control group. Our results were in agreement with the results obtained by ***(17)*** who showed that destruction of leydig cells may cause testicular atrophy; gondal dysfunction, erectile dysfunction, and

male factor infertility. The testosterone hormone level in this study is markedly decreased. Our results were in agreement with the results obtained by ***(9)***, ***(15), (6)*** who reported that serum level of testosterone was significantly decreased in a dose dependent manner when compared with those of the control rats. In disagreement with the results obtained by ***(18)***, ***(19)*** who have reported that decreasein testosterone level was attributed toincreased activity of hydroxylase, an enzyme known to increase testosterone level metabolism. In this study nicotine withdrawal results in improvement of the testicular histology in the form of complete spermatogenesis with normal spermatogonia, primary spermatocytes, spermatids and spermatozoa in addition to prominent improvement of serum testosterone level. This is in agreement with ***(15)*** who reported that there were both regeneration of the germinal epithelium and restructuring of the interstitum towards normal in the recovery groups. The results suggest that nicotine has deleterious effect on the male reproductive organ of albino rats ameliorated by nicotine cessation. Our study demonstrated that vitamin E results in improvement of the testicular histology in the form of complete spermatogenesis with normal spermatogonia, primary spermatocytes, spermatids and spermatozoa in addition to prominent improvement of serum testosterone level. This is in agreement with***(20)***, ***(9)*** who revealed that coadministration of vitamin E with nicotine protected the degeneration of different generations of germ cells to some extent and significantly increased its number toward control**,** but our study added that testestrerone level has been increased. In agreement with ***(9)*** vitamin E improved the reduction in sperm characteristics, hormone levels and testicular alterations observed in nicotine treated rats. The study shows that nicotine exerts significant deleterious effects on male reproductive system and the concurrent administration of vitamin E ameliorated these detrimental effects. We accept the opinion of (***21)*** who had reported that nicotine and nicotine based products should therefore be taken with caution in cases of infertility as his results run in the same way with our results. In the present study the combined treatment of nicotine and vitamin E caused increase in the testosterone level and improvement of the histological and immunohistochemical changes in the testicular tissue. This was almost, also, observed in animals groups after withdrawal of nicotine. The ameliorating effect of vitamin E noticed in the present study may be attributed to its antioxidant properties as reported by ***(22).*** Natural antioxidants as polyphenols of vitamin E have received much attention for treatment of oxidative-stress-related pathological conditions ***(7). (8)*** reported that vitamin E is a rich source of polyphenols, which are antioxidants having an ameliorating effect in genital organs. ***(23)*** suggested that the ameliorating effect of vitamin E on Leydig cells was due to its antioxidant activity.

Conclusion

The outcome of this study shows that the toxic effect of nicotine was improved by nicotine withdrawal and concomitant administration vitamin E, so, it is advisable to stop smoking as early as possible for avoiding irreversible damage of the testicular tissue. It was also clear from the results of the study that smoking has a greatly deteriorating effect on reproduction which can be reduced by vitamin E.

References

1. Rubes, J.A., Lowe, D.X., Moore, U.D., Slott, Y.V. and Evenson, D.L., (1998): Smoking Cigarette is associated with increased sperm disomy in teenager men, Fertil. Steril., 70; pp. 715.
2. Ramage, L.A., Jones, A.C., Whelan, C.J., (2006): Re_induction of apoptosis with tobacco smoke and related products in A549 lungepithelial cells in *vitro*. J. Inflamm. (Lond),3,3.
3. Alberg, A.J., (2008): Cigarette smoking: health effect and control strategies. Drugs Today (Barc), 44(12):895-90.
4. Sadeu, J.C., Hughes, C.L., Agarwal, S.H. and Foster, W.G., (2010): Alcohol, drugs, caffeine, tobacco and environmental contaminant exposure: reproductive health consequences and clinical implications. Crit. Rev. Toxicol., 40; pp. 633-652.
5. Reddy, S.K., Londonkar, R.T. and Patil, S.B., (1998): Testicular changes due to graded doses of nicotine in albino mice. Ind. J. Physiol. Pharma. 42; pp. 276-280.
6. Kavitharaj, N.K. and Vijayammal, P.L., (1999): Nicotine administration induced changes in the gonadal functions in malerats. Pharmacology; 58: pp. 2-7.
7. Yokozawa, Y.T., Rhyu, D.Y. and Cho, E.J., (2004): Epigallocatechin-3-O-gallate ameliorates the damages related to peroxynitrite production by mechanism distinct from those of other free radical inhibitors. J. Pharma Pharmacol., 56; pp. 231-239.
8. Ogura, R.A., Ikeda N.L., Yuki, K.D., Morita, R.O., Saiga, K.S., Blackstock, C.B. and Kasamatsu, J.T., (2008): Genotoxicitystudies on nicotine Chemical Toxicology; 46;pp. 2190-2200.
9. Oyeyemi, W.A., Shittu, S.T., Kolawole, T.A. and Akinola, A.O., (2015): Protective Effect of Vitamin E on Nicotine Induced Reproductive Toxicity in Male Rats. Igbinedion University.

Available from: http://www.ajol.info/index.php/njbas/index 23(1); pp. 7-13 [5june 2015].
10. Crocker, J.U., Boldy, Q.D. and Egan, M.J., (1989): How should we count AgNORs? Proposal for a standardized approach. J Pathol 198 (158): pp. 185-88.
11. Hansel, W.U. and Convey, E.M., (1983): Physiology of the esterous cycle. J. Animal Sci., 2th edition. Chapter 5: pp. 404-424.
12. Newberne, J.W., Kuhn, W.L. and Elsea, J.R., (1996): Toxicologic studies on nicotine. Toxicology and pharmacology 9; pp. 44-56.
13. Brown, J.L. and Chakraborty, P.K., (2001): Characterization of the effects of nicotine on reproductive physiology in male rats of various ages. Acta. Endocrino. Copenh. Jul. 118 (3): pp. 437-43.
14. Rajpurkar, A.L. and Dhabuwala, C.B., (2000): Morphometric analysis of rat testis following chronic exposure to cigarette smoke.J Environ PatholToxicolOncol 19; pp. 363-368.
15. Oyeyipo, I.P., Raji, A.Y., Emikpe, B.O. and Bolarinwa, A.F., (2010): Effects of Oral Administration of Nicotine on Organ Weight, Serum Testosterone Level and Testicular Histology in Male Rats. Nig. J. Physiol. Sci. 25(10); pp. 81– 86.
16. Nelson, W.O. and Patanelli, D.J., (1998): Normal and abnormal germinal cells of rats following administration of nicotine. Fed. Proc. 21; pp. 437.
17. Kim, I.S., Ariyaratne, H.B. and Mendis, S.M., (2005): Changes in the Testis Interstitium of Brown Norway Ratswith Aging and Effects of nicotine on the Aged Testes in Enhancing the Steroidogenic Potential. BiolReprod., 66(5); pp. 1359- 1366.
18. Wang, H.L., Li, D.Y. and Cho, C.U., (2000): Exposure to cigarette smoke increases apoptosis in the rat gastric mucosa through a reactive oxygen spices-mediated and p53-independent pathway. Free Radic. Biol, Med., 28(7); pp.1125-1131.
19. Mittler, J.C., Pogach, G.L. and Ertel, N.K., (1983): Effects of chronic smoking ontestosterone metabolism in dogs. Journal of Steroid Biochemistry, 18; pp. 759–763.
20. Kuladip, J.D., Prabhat, K.S. and Dipak, K.D., (2016): Nicotine Diminishes Testicular Gametogenesis, Steroidogenesis, and Steroidogenic activity in Adult Albino Rats. West Bengal University of Animal and Fishery Sciences. Available from: http://toxsci.oxfordjournals.org [4 august 2016].
21. Egesie, U.G., Nanyak, Z.g., Ibraheem, M.G., Agbowuro, A.A. and Gomerep, S.S., (2013): Evaluation of andrological indices and testicular histology following administration of varied doses of nicotine. Journal of Biology, Agriculture and Healthcare. 167: pp. 570-579.
22. Patra, S.K., Rizzi, F.L., Silva, A. M., Rugina, D.O. and Bettuzzi, S.H., (2008): Molecular Targets of Epigallocatechin-3-gallate (EGCG): Specificity and interaction with memberane lipid rafts. J PhysiolPharmacol., 59; pp. 217-235.
23. Hakim, I.A., Harris, R.B., Brown, P.S., Chow, H.H. and Wiseman. S.D., (2003): Effect of increased vitamin E consumption on oxidative DNA damage among smokers: a randomized controlled study. J Nutr 133: pp. 3303S-3309S.

2/23/2017