IJPSR (2015), Vol. 6, Issue 6 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 16 October, 2014; received in revised form, 17 December, 2014; accepted, 08 February, 2015; published 01 June, 2015

COMBINED EFFECTS OF ATORVASTATIN AND NICOTINE ON TESTICULAR EXPRESSION OF STEROIDOGENIC ENZYMES IN EXPERIMENTAL RATS

Syam Das S and M Indira*

Department of Biochemistry, University of Kerala, Kariavattom, Thiruvananthapuram-695 581, Kerala, India.

Keywords:

Atorvastatin, Nicotine, Steroidogenic enzymes, Testosterone

Correspondence to Author:
M. Indira

Department of Biochemistry, University of Kerala, Kariavattom Thiruvananthapuram - 695 581 Kerala, India.

E-mail: indiramadambath@gmail.com

ABSTRACT: Nicotine and atorvastatin have indirect effects on reproductive function. So this study focused on changes in testosterone biosynthesis in rats co administered nicotine and atorvastatin. Rats were divided into 4 groups.(1) Control, (2) Atorvastatin (10 mg/Kg b.wt), (3) Nicotine (0.6 mg/Kg b.wt)and (4) Atorvastatin (10 mg/Kg b.wt) + nicotine (0.6 mg/Kg b.wt). After sixty days of treatment testicular cholesterol level, serum testosterone level, activity of HMG CoA reductase in the liver, activities and expressions of testicular steroidogenic enzymes; 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD)and peroxidation products were assayed. Nicotine administration caused hypercholesterolemia, decreased expression of steroidogenic enzymes and testosterone levels. Administration of atorvastatin reduced cholesterol levels, decreased the expression and activity of steroidogenic enzymes and testosterone levels. Co-administration of nicotine and atorvastatin enhanced the testosterone level in comparison with nicotine administration. Enhanced biosynthesis of testosterone in coadministered group may be due to increased activities and expressions of 3β-HSD and 17β-HSD and enhanced expression of StAR protein indicating enhanced transportation of cholesterol. Reduction in oxidative stress was also a contributory factor. In short, both nicotine and atorvastatin independently caused testicular toxicity. But their co- administration reduced the testicular toxicity induced by nicotine.

INTRODUCTION: Testosterone, a 19-carbon steroid secreted by the testis, is the predominant androgen in most mammalian species. Testosterone plays a critical role in mammalian reproduction. It is essential for maintaining sexual function, germ cell development, and accessory sex organs. It is produced in the testis by a heterogeneous group of cells that includes the adult Leydig cells. Testosterone secretion by Leydig cells is under the control of Luteinizing Hormone.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(6).2511-17

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(6).2511-17

The precursor for testosterone synthesis is cholesterol and so the rate-limiting step in testosterone biosynthesis is the delivery of cholesterol to the inner mitochondrial membrane. This is the site of the cholesterol side chain cleavage complex that converts cholesterol to pregnenolone. A steroidogenesis acute regulatory protein (StAR) makes cholesterol available to the cholesterol side chain cleavage complex (Cyt P450scc) and regulates the rate of testosterone biosynthesis [1].

At the inner mitochondrial membrane, cholesterol associates with the Cyt P450scc enzyme, and is converted to pregnenolone through a series of hydroxylation reactions [2]. Pregnenolone is translocated from the mitochondria to the smooth endoplasmic reticulum (SER) where it is converted

to testosterone by the actions of two important enzymes; 3β-hydroxysteroid dehydrogenase (3β-HSD) and17β-hydroxysteroid dehydrogenase (17β-HSD).

Nicotine; the principal alkaloid in tobacco is reported to enhance the synthesis of cholesterol, triglycerides, phospholipids and free fatty acids in the testes and increases peroxidative damage [3]. Studies have shown that it is a testicular toxin [4]. Statins are well known HMG CoA inhibitors used to treat hypercholesterolemia. It is reported that monthly statin prescriptions have increased from 45.8 to 84.1 per 1,000 patients with coronary heart disease. The proportion of the Indian population receiving a defined daily statin dose increased from 3.35 percent to 7.78 percent [5]. Independent of their lipid lowering properties, statins have been shown to exert pleiotropic effects on endothelial function, vascular inflammation, immunomodulation, bone mineralization etc [6]. *In vitro* studies with simvastatin have been found to reduce testosterone production[7].

Since cholesterol is the precursor of testosterone an optimum amount of cholesterol is essential for the normal reproductive function. Nicotine induces hypercholesterolemia and statin reduces cholesterol levels. So we were interested to study how the drug-drug interaction of nicotine and statin would affect the testicular expression of steroidogenic enzymes in experimental rats. In our study we focused on the changes in testosterone synthesis pathway of male rats co administered with atorvastatin and nicotine.

MATERIALS AND METHODS:

Male albino rats (Sprague Dawley strain, average weight of 175 ± 25gm) were selected and housed in polypropylene cages. The cages were kept in a room at 28°C-32°C. The light cycle was 12h light and 12h dark. Animals were handled as per the laboratory animal welfare guidelines.

Groups:

Animals were divided into four groups as follows:

Group (C) : Control fed with normal diet

Group (A) : Atorvastatin (10mg/ 1Kg body wt/day).

Group (N) : Nicotine (0.6mg/kg body wt/day).

Group (N+A) : Atorvastatin + Nicotine (10mg/ Kg body wt /day + 0.6mg/kg body wt/day)

Atorvastatin (Sigma-Aldrich, St. Louis, MO, USA) was freshly dissolved in distilled water and given orally by gastric tube. Nicotine was injected intraperitonially. The dose of the nicotine was selected from previous studies [8]. Rats were fed with standard laboratory diet supplied by Ashirwad Pvt Ltd., India and water was given *ad libitum.* The duration of the experiment was 60 days. Animal experiments were approved by the Institutional Animal Ethics Committee [IAEC No-KU-25/2011-BC-MI (31)]. At the end of the experiment period (60days), the animals were sacrificed after overnight fasting. Then testes and epididymis were dissected out and cleaned with ice-cold saline, blotted dry and immediately transferred to ice-cold containers for various biochemical evaluations. Blood was collected and testosterone content was analysed using separated serum.

Biochemical assays:

Activity of HMG CoA reductase was determined in liver by the method Rao and Ramakrishnan [9]. Testicular cholesterol was estimated by the method of Zak et al[10]. Activities of 3β-HSD and 17β-HSD was determined by the method of Shivanandappa and Venkatesh, and Jarabak et al respectively [11, 12]. Total serum testosterone was estimated by the method of RIA using the kit for Coat-A-count purchased from Diagnostic Product Corporation, USA (Catalogue No: LKTT). Malondialdehyde was estimated by the method of Ohkawa and hydroperoxides were estimated by the iodometric method of Mair and Hall [13, 14]. Conjugated dienes were estimated according to the method of Recknagel and Ghoshal[15].

mRNA Expression studies:

Total RNA was isolated from the testes using TRI reagent (Sigma Aldrich) by the method described by Chomczynski and Sacchi[16].

Quantification of 3β HSD, 17β HSD and StAR protein:

The isolated RNA was used for Reverse Transcriptase-Polymerase Chain reaction (RTPCR) to quantify the expression of 3β-HSD, 17β-HSD

and Steroidogenic Acute Regulatory Protein (StAR protein). Total RNA was reverse transcribed and PCR was performed using Eppendorf RT-PCR kit with gene-specific primers. Sequence of the primers is given in **Table 1**. PCR mixture was resolved on 2% agarose gel containing ethidium bromide. Then the gels were subjected to densitometric scanning (Bio-Rad Gel Doc, California, USA) to determine the OD of each and then normalized against an internal control, Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using Quantity One imaging software.

TABLE 1: SEQUENCE OF PRIMERS

Gene product	Oligonucleotide	Accession No:
3 β-HSD	Senseprimer:5-TCTGAAAGGTACCCAGAACCTATT-3 Anti-sense primer:5-TTGCTTGAACACAGGCCTCCA-3	Genebank:L17138
17β-HSD	Senseprimer:5-AGTGTGGGAGGCTTGATGGGA-3 Anti-senseprimer:5-CACTTCGTGGAATGGCAGTCC-3	Genebank:NM012851
StAR	Senseprimer:5-GAGCTCTCTGCTTGGTTCTCA-3 Anti-senseprimer:5-TTGAGTATGCCCAAGGCCTT-3	Genebank:EF091859
GAPDH	Senseprimer:5-GAAGGGCTCATGACCACAGT-3 Anti-sense primer:5-GGATGCAGGGATGATGTTCT-3	Genebank:NG028301

Histopathological Studies:

Testes were kept in 10% formalin for 2 weeks, then dehydrated and embedded in paraffin blocks. Each paraffin block was sectioned into 5µm thickness stained with hematoxylin and eosin, and evaluated histologically using a light microscope and an image analyser (Leica Application Suit, Leica Microsystems, India). Each tissue section was assessed for histological changes such as epithelial atrophy, interstitial oedema and vacuoles of the epithelial cells.

Statistical Analysis:

Statistical analyses were carried out using the Statistical Package for Social Science (SPSS Inc. Chicago, IL, USA) version 17.0. Data with normal distribution and homogenous variance were analysed using One-way analysis of variance (ANOVA). Pair fed comparisons between the groups was made by Duncan's multiple range test $p \leq 0.05$ was considered to be significant.

RESULTS:

Activity of HMG CoA reductase:

The HMG CoA/Mevalonate ratio was measured. Lower ratio indicates higher enzyme activity. The activity of HMG CoA reductase was significantly decreased in atorvastatin treated group (**Fig.1**) in comparison to the control group. Significantly higher activity was seen in the nicotine treated group when compared to atorvastatin and control groups. Co administration of nicotine with statin decreased the activity in comparison to the nicotine group.

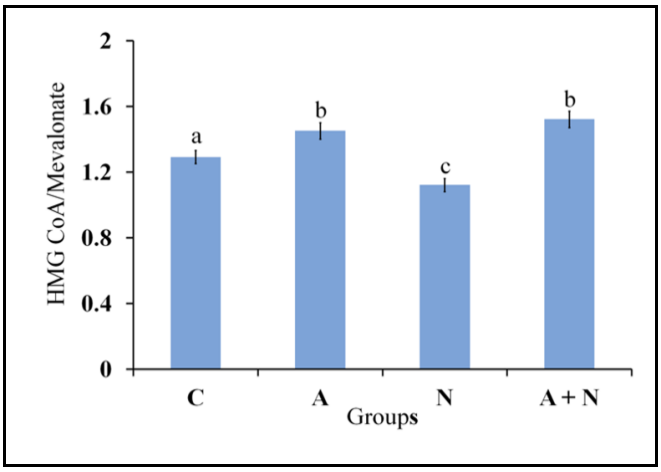


FIG.1: ACTIVITY OF HMG CoA REDECTASE

C: Control, A: Atorvastatin, N: Nicotine and A+N: Atorvastatin +Nicotine. Values are expressed as mean ±SEM of six rats in each group. Values not sharing a common superscript differ significantly at $p < 0.05$

Concentration of Cholesterol in testes:

There was a significant increase in the concentration of testicular cholesterol in nicotine administered group when compared to control group (**Fig.2**). Atorvastatin administered group showed significant decrease in the concentration of cholesterol over the control group. A+N group showed decrease in cholesterol level compared to

nicotine treated group but no significant change in comparison with control group.

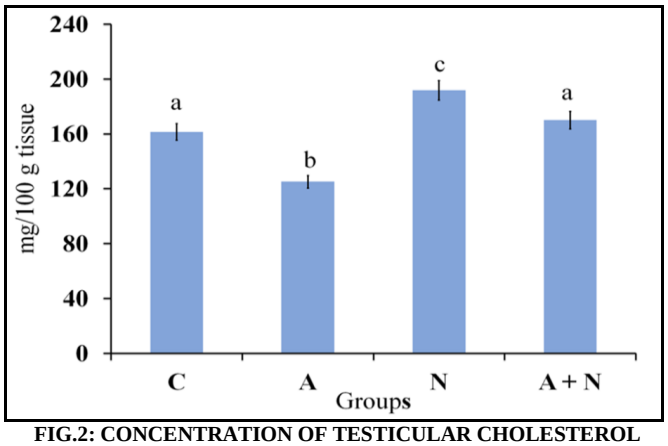


FIG.2: CONCENTRATION OF TESTICULAR CHOLESTEROL

C: Control, A: Atorvastatin, N: Nicotine and A+N: Atorvastatin +Nicotine. Values are expressed as mean ±SEM of six rats in each group. Values not sharing a common superscript differ significantly at p< 0.05

Activities of 3β-HSD and 17β-HSD in testes:

The activities of 3β-HSD and 17β-HSD were found to be significantly decreased in atorvastatin and nicotine administered group (**Fig.3**) when compared to the control group. But Co administration of statin and nicotine enhanced the activities of 3β-HSD and 17β-HSD in comparison with nicotine group.

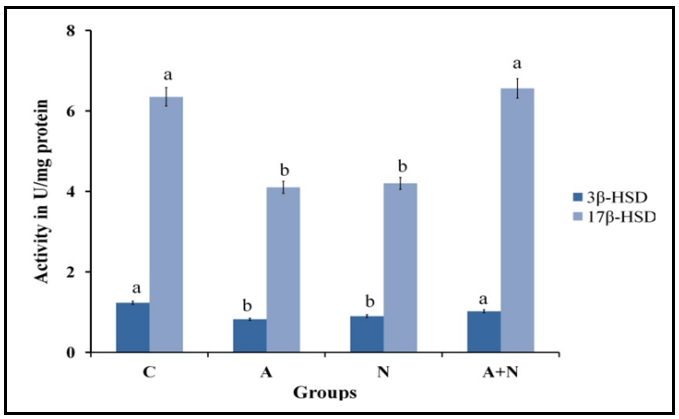


FIG. 3: ACTIVITIES OF 3β –HSD AND 17β–HSD IN TESTIS

C: Control, A: Atorvastatin, N: Nicotine and A+N: Atorvastatin +Nicotine. Values are expressed as mean ±SEM of six rats in each group. Values not sharing a common superscript differ significantly at p< 0.05

RT-PCR analysis of 3β-HSD, 17β-HSD and StAR:

The mRNA expressions of 3β-HSD, 17β-HSD and StAR protein were significantly decreased in both atorvastatin and nicotine treated groups compared to control (**Fig.4**). Co administration of statin and nicotine reduced mRNA expressions when compared to the control group.

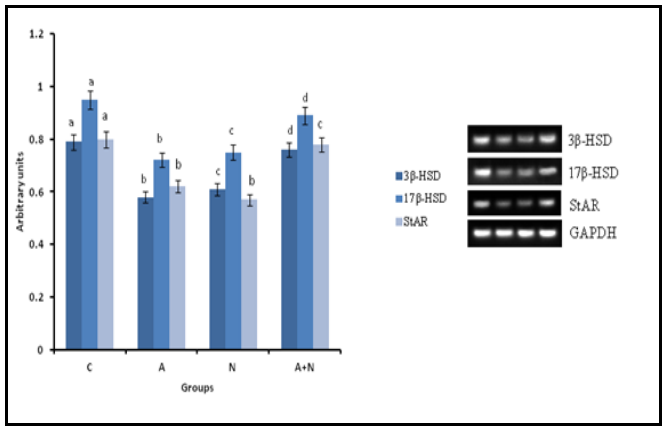


FIG.4: mRNA EXPRESSIONS OF 3β–HSD, 17β–HSD AND StAR PROTIEN

Expressions of 3β-HSD, 17β-HSD and StAR protien were analysed in the cytoplasmic fraction of testis by agarose gel electrophoresis and the intensities of the bands were compared with that of the intensities of Glyceraldehyde 3-Phospho Dehydrogenase bands expressed in the samples. Intensities of the bands were quantified using Biorad gel doc and plotted. The results presented are average of quadruplicate experiments, ± SEM statistically significant at P<0.05

Concentration of total testosterone in serum:

Total testosterone level in serum was decreased significantly in atorvastatin, nicotine and co administered groups (**Fig.5**) compared to control. But administration of nicotine along with statin increased testosterone levels when compared to nicotine group but decreased when compared to the control group.

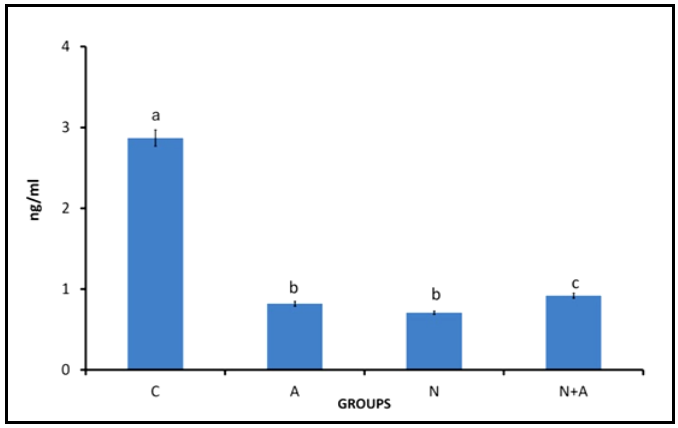


FIG.5: CONCENTRATION OF TOTAL TESTOSTERONE IN SERUM

C: Control, A: Atorvastatin, N: Nicotine and A+N: Atorvastatin +Nicotine. Values are expressed as mean ±SEM of six rats in each group. Values not sharing a common superscript differ significantly at p< 0.05

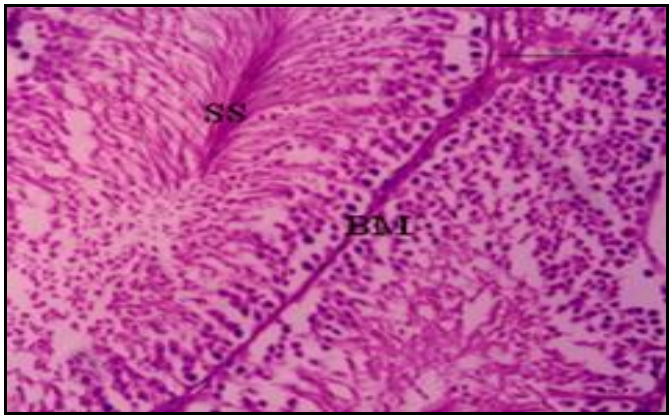
Lipid peroxidation Products:

Lipid peroxidation products (Hydroperoxides, and Conjugated dienes) were significantly increased in atorvastatin treated group compared to control (**Table 2**). Nicotine treated group showed the maximum increase in comparison with control, atorvastatin and co administered groups. Co administration with atorvastatin significantly decreased peroxidation products compared to nicotine treated group. MDA levels did not show any significant change in atorvastatin and co administered group compared to control.

TABLE 2: CONCENTRATION OF LIPID PEROXIDATION PRODUCTS IN TESTES

Groups	Hydroperoxides (mM/100g wet tissue)	Conjugated diens (mM/100g wet tissue)	Malondialdehydes (mM/100g wet tissue)
C	4.18 ± 0.15^{a}	43.39 ± 1.61^{a}	0.68 ± 0.02^{a}
A	5.12 ± 0.19^{b}	52.08 ± 1.94^{b}	0.74 ± 0.02^{a}
N	13.15 ± 0.48^{c}	94.74 ± 3.52^{c}	0.96 ± 0.03^{b}
N+A	7.08 ± 0.26^{d}	59.39 ± 2.21^{d}	0.80 ± 0.02^{a}

Values are expressed as mean ±SEM of six rats in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$



(a)

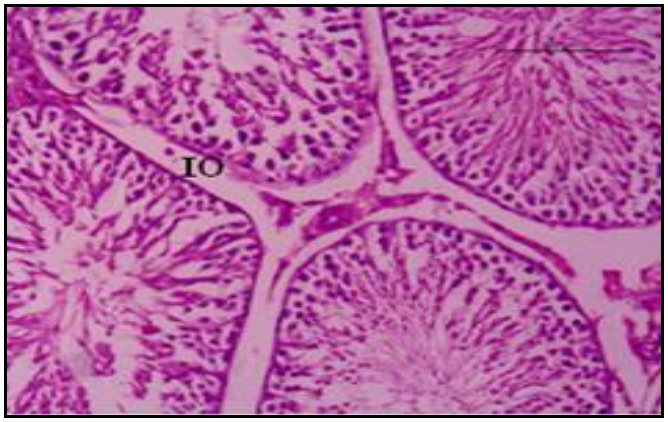
FIG: 6(a) MICROGRAPH OF TESTES OF THE CONTROL GROUP ORIGINAL MAGNIFICATION (40x)

This slide shows the structure of normal testis. Each testicular capsule is surrounded by a basement membrane. Testicular capsules consist of germ cells that mature to form sperms in the lumen. SS: Seminiferous tubule containing sperms, BM: Basement membrane

(b)

FIG: 6(b) MICROGRAPH OF TESTES OF THE ATORVASTATIN TREATED GROUP ORIGINAL MAGNIFICATION (40x)

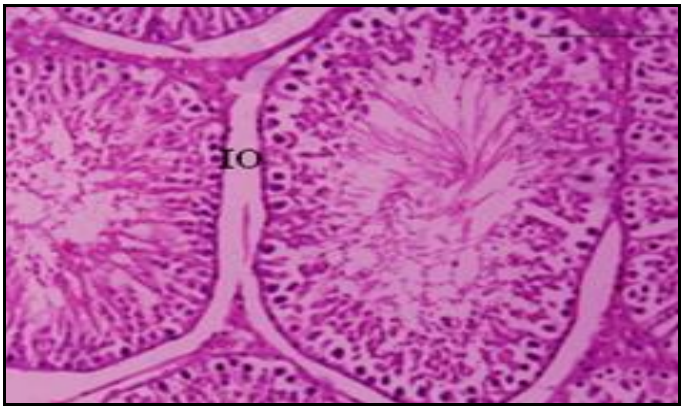
But atorvastatin treated rats show interstitial oedema but no other signs of testicular damage have been found. IO: Intestetial oedema



(c)

FIG: 6(c) MICROGRAPH OF TESTES OF THE NICOTINE TREATED GROUP ORIGINAL MAGNIFICATION (40x)

This slide show loosely packed germ cells with few cells attached to basement membrane. The cells are clustered without cytoplasm. Less numbers of matured sperms can be observed. DC: Detached cells from Basement membrane



(d)

FIG: 6(d) MICROGRAPH OF TESTES OF THE ATORVASTATIN + NICOTINE GROUP ORIGINAL MAGNIFICATION (40x)

Co administered group shows only interstitial oedema and seminiferous tubule containing normal sperms. Less number of apoptotic cells compared to nicotine treated group. IO: Intestetial oedema

DISCUSSION: Testosterone is the key hormone that plays a significant role in male reproductive function. In this study we focused on the influence of co administration of nicotine and atorvastatin on the biosynthesis of testosterone. Low levels of testosterone were observed in the statin and nicotine treated groups. This is due to the suppression of its biosynthesis since both activities and mRNA expressions of 3β-HSD and 17β-HSD were lower in these groups.

Cholesterol is the precursor for the synthesis of testosterone. In agreement with the previous studies [17, 18] its level was reduced in the statin administered group (**Fig. 2**). This was due to the inhibition of HMG CoA reductase activity. This may also be a contributory factor for the reduced biosynthesis of testosterone in the statin group. As reported nicotine administration resulted in significantly increased activity of HMG CoA and increased level of testicular cholesterol [7]. Co administration of atorvastatin reduced cholesterol level induced by nicotine by inhibiting HMG CoA reductase.

Delivery of cholesterol to the inner mitochondrial membrane is the rate-limiting step in steriodogenesis [19]. Therefore regulation of testosterone synthesis must be controlled by factors involved in translocation of cholesterol. A range of proteins have been implicated including START domain proteins such as MLN64 and StAR with roles in targeting cholesterol to the mitochondria [20]. So we had analysed the mRNA expression level of StAR protein in testis and it was found to decrease significantly both in atorvastatin and nicotine group when compared to control group (**Fig.4**).

This is in line with previous studies [21]. It had been proved that the level of StAR protein in the Leydig cells significantly affects the testosterone production[22]. Co administration of nicotine and statin enhanced the expression of StAR protein in comparison with nicotine indicating increased delivery of cholesterol for testosterone biosynthesis. This supports the enhanced cholesterol and testosterone levels in the coadministered group. Cholesterol biosynthesis was enhanced in the nicotine group as evidenced by the increased activity of HMG CoA reductase (**Fig. 1**) and elevated level of cholesterol. But this cholesterol was not channelled for the production of testosterone due to the reduced activities of 3β-HSD and 17β-HSD. This is in agreement with the previous studies of Seema *et al* that nicotine suppresses testicular androgenic enzyme [8]. We also observed significantly increased lipid peroxidation products in nicotine treated group compared to control. It is well known that nicotine can induce increased production of reactive oxygen species and thereby induce testicular oxidative stress [3]. In co administered group atorvastatin reduced lipid peroxidation products in comparison with nicotine group (**Table 2**).

Our histopathological studies support our biochemical findings. The testicular alterations observed in atorvastatin and nicotine treated rats may be due to reduced testosterone levels since testosterone is needed for the normal morphology of testis. Aydos *et al* [23] have also observed morphological changes in the testis on nicotine administration. In agreement with our biochemical studies the testicular architecture of co administered group showed lesser toxicity in comparison with either nicotine or statin alone treated groups (**Fig.6**).

CONCLUSION: In conclusion co administration ameliorates the toxic effect induced by nicotine to some extent by up regulating the testosterone production and by reducing the oxidative stress. The drug-drug interaction is an interesting field of study. The interaction of nicotine and atorvastatin might have reduced the bioavailability of nicotine and thus reducing its toxicity. But only further studies can confirm it.

ACKNOWLEDGEMENT: Financial assistance in form of Research Fellowship from District Development Office for SC, Trivandrum, Kerala is gratefully acknowledged.

CONFLICT OF INTEREST STATEMENT: The authors declares that there is no conflict of interest.

REFERENCES:

1. Marc Luetjens C and Gerhard FW: "Testosterone: Action, Deficiency, Substitution" Eberhard N, Hermann MB & Susan N (eds). Cambridge University Press, Cambridge, UK; Fourth Edition 2012: 16-19

2. Hall PF: "The Roles of Cytochromes P-450 in the Regulation of Steroidogenesis", Comprehensive Physiol, 2011; 1(7): 413–437.
3. Li S, Lu D, and Zhang Y: "Long-term treatment of hydrogen-rich saline abates testicular oxidative stress induced by nicotine in mice". J. Assist Reprod. Genet, 2014; 31(1): 109-14.
4. Oyeyipo IP, Raji Y, Bolarinwa AF: "Nicotine alters male reproductive hormones in male albino rats: The role of cessation", J. Hum. Reprod. Sci. 2013; 6 (1):40-4.
5. Choudhry NK, Dugani S, Shrank WH, Polinski JM, Stark CE, Gupta R, Prabhakaran D, Brill G, Jha P: "Despite increased use and sales of statins in India, per capita prescription rates remain far below high-income countries", Health Aff.2014 ;33(2):273-82
6. Tandon V, Bano G and Khajuria V: "Pleiotropic effects of statins", Educational. Forum 2004; 37(2): 77-85.
7. Antony GH, Jos J and Theo JB: "The HMG CoA reductase inhibitor simvastatin suppresses human testicular testosterone synthesis in vitro by a selective inhibitory effect on 17 ketosteroid oxidoreductase enzyme activity". J. Steroid Biochem. Mol. Biol 1991; 38(4): 465-8.
8. Seema P, Swathy SS, and Indira M: "Protective effect of selenium on nicotine-induced testicular toxicity in rats", Biol. Trace Elem. Res 2007; 120:212–218.
9. Rao AV and Ramakrishnan S: "Estimation of HMG CoA reductase activity", Clin.Chem 1975; 21(10): 1523–5.
10. Zak B and Ressler N: "Methodology in determination of cholesterol; a review", Am. J. Clin. Pathol 1955; 23(4): 433–446.
11. Shivanandappa T and Venkatesh SA: "Colourimetric assay method for 3 beta hydroxyl delta steroid dehydrogenase", Anal. Biochem1997; 254(1): 57-61.
12. Jarabak J: "Soluble 17 beta hydroxyl steroid dehydrogenase of human placenta". Methods in enzymology. Raymond BC (ed). Academic Press. New York, 1969, Vol- 15, pp.746-52.
13. Ohkawa H, Ohishi N and Yagi K: "Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction", Anal. Biochem 1979; 95(2): 351–358.
14. Mair RD and Hall T: "Determination of organic peroxides by physical chemical and colorimetric methods", In: Swern D & Wiley CD (eds). Inorganic peroxides II. Wiley, Intersciences. New York, 1971 Vol-2, pp. 535-38.
15. Recknagel RO and Ghoshal AK: "Quantitative estimation of peroxidative degeneration of rat liver microsomal and mitochondrial lipids after carbon tetrachloride poisoning", Exp. Mol. Pathol 1966; 5(5): 413–426.
16. Chomcynski P and Sacchi N: "Single-step method of RNA Isolation by Acid guanidinium thiocyanate-phenol-chloroform Extraction", Anal. Biochem 1987; 162(1): 156-9.
17. Lee HY, Cho HJ, Kim HY, Jeon HK, Shin JH, Kang SM, Baek SH:"Effects of intensive versus mild lipid lowering by statins in patients with ischemic congestive heart failure: Korean pitavastatin heart failure (SAPHIRE) study", Korean J. Intern. Med. 2014; 29(6):754-63.
18. Lubica C, Hyspler R, Micuda S, Cibicek N, Zivna H, Jun D, Ticha A, Brcakova E, and Palicka V: "The influence of simvastatin, atorvastatin and high-cholesterol diet on acetyl cholinesterase activity, amyloid beta and cholesterol synthesis in rat brain", Steroids 2009;74(1): 13-19
19. Rone MB, Fan JJ and Papadopoulos V: "Cholesterol transport in steroid biosynthesis: Role of protein-protein interactions and implications in disease states", Biochemica et Biophysica Acta 2009; 1791(7): 646-58.
20. Kuladip J, Prabhat KS and Dipak KD: "Nicotine Diminishes Testicular Gametogenesis, Steroidogenesis, and Steroidogenic acute regulatory protein Expression in adult Albino rats: Possible influence on pituitary gonadotropins and Alteration of testicular antioxidant status", Toxicol. Sci 2010;116(2): 647–659
21. Bose M, Debnath D, Chen Y and Bose HS: "Folding, activity and import of steroidogenic acute regulatory protein into mitochondria changed by nicotine exposure". J. Mol. Endocrinol 2007; 39: 67–79.
22. Raucci F, D' Aniello A, Di Fiore MM:"Stimulation of androgen production by D-aspartate through the enhancement of StAR, P450scc and 3β-HSD mRNA levels in vivo rat testis and in culture of immature rat Leydig cells, "Steroids. 2014; 84:103-10.
23. Aydos K, Guven MC, Can B and Ergun A: "Nicotine toxicity to the ultra-structure of the testis in rats", BJU International 2001; 88: 622-626.

How to cite this article:
Das SS and Indira M: Combined Effects of Atorvastatin and Nicotine on Testicular Expression of Steroidogenic Enzymes in Experimental Rats. Int J Pharm Sci Res 2015; 6(6): 2511-17.doi: 10.13040/IJPSR.0975-8232.6(6).2511-17.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)