

Differential effects of chronic nicotine administration on markers of oxidative stress and cellular damage in male and female rats

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Abstract: Most studies are gender blind regarding the effects of chronic nicotine administration on oxidative stress in brain and liver. So we designed this study to determine the differential effects of chronic nicotine administration on parameters of oxidative stress and antioxidant status in brain and liver of male and female rats as well as on liver function tests (LFTs) and lipid profile parameters. Catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and malondialdehyde (MDA) in brain and liver were assessed in controls and nicotine treated male and female rats. Two-way analysis of variance and Pearson's correlation coefficient was applied. Raised GSH, CAT and SOD and less MDA levels in the brain, lowered liver GSH and lipid profile but raised LFTs in females as compared to male rats were found. Significant inverse correlations were found between serum triglycerides versus both liver and brain GSH and between serum HDL versus brain MDA in nicotine treated male rats. In conclusion, oxidative stress was found to be decreased in the brain but increased in the liver in females compared to males. So the females suffered more from oxidative stress related damage to the liver cells.

Keywords: Antioxidant enzymes, lipid peroxidation, liver function tests, nicotine, oxidative stress.

INTRODUCTION

Nicotine, the most important psycho active agent present in tobacco, could induce the formation of free radicals resulting in oxidative stress that in turn is responsible for the development of various pathologies such as cardiovascular diseases, brain diseases and hepatic disorders (Mahrous *et al.*, 2019). Smoking rates in women as compared to men are continuously rising throughout the world (Jafari *et al.*, 2021) and studies have reported women smokers to be more prone to have raised levels of blood and urinary markers of oxidative stress as compared to men. Hakim *et al.* has depicted higher levels of urinary markers of oxidative deoxyribonucleic acid (DNA) and lipid damage in female current smokers as compared to their male counterparts (Hakim *et al.*, 2012). Additionally, another study conducted on 140 subjects has also depicted significant rise in the levels of anti-5-hydroxymethyl-2'-deoxyuridine autoantibody in females as compared to male heavy smokers (La Verne *et al.*, 2001). However, most studies are gender blind regarding the differential effects of chronic nicotine administration on parameters of oxidative stress and antioxidant status in brain and liver of male and female rats.

When smoked, nicotine gets rapidly absorbed into the blood and metabolized in the liver (Benowitz *et al.*, 2016) where it has been shown to exert various detrimental effects through induction of oxidative stress (Simeonova *et al.*, 2014). The free radical production results in attack on membrane lipids and formation of Malon dialdehyde (MDA) and lipid peroxidation products. Raised levels of MDA in blood (Joshi *et al.*, 2020) and liver tissue in

rodents exposed to nicotine has been assessed in various studies (Ashakumary and Vijayammal, 1996; Zhang *et al.*, 2001). Additionally, reduced antioxidant defense secondary to nicotine use evident by greatly reduced levels of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and catalase in the liver has been reported by some authors (Micheva *et al.*, 2009; Simeonova *et al.*, 2012) but very few studies have reported the differences based on gender. Mammalian brain has been shown to have high oxygen demand due to high metabolic activity, which encourages the generation of free radicals (Ashok *et al.*, 2022). Additionally, the high concentration of unsaturated lipids as well as redox- active transition metals and a significantly poorer antioxidant defense owing to low levels of antioxidant enzymes makes the neurons more susceptible to suffer from oxidative stress (Olmez and Ozyurt, 2012). Oxidative stress and mitochondrial dysfunction underlie the development and progression of various brain pathologies and could be induced by administration of nicotine (Elsonbaty and Ismail, 2020; Das *et al.*, 2009). However, there is a scarcity of studies depicting the gender differences in the effect of chronic administration of nicotine on the development of oxidative stress in brain.

Nicotine induced oxidative stress has been proposed to be of paramount importance in the pathogenesis of various cardiovascular diseases such as atherosclerosis (Ramalingam *et al.*, 2021). Many studies have reported an atherogenic lipid profile in smokers (Nakamura *et al.*, 2021) with low levels of High Density Lipoprotein Cholesterol (HDL-C) and enhanced concentrations of Low Density Lipoprotein Cholesterol (LDL-C) in smokers versus non-smokers (Taiwo and Thanni, 2021;

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Cichosz *et al.*, 2020). However, few studies have been conducted to ascertain the gender differences in relation to any alterations in lipid profile consequent to nicotine use.

Hence, we designed this study to determine the differential effects of chronic nicotine administration on tissue antioxidant status by assessing the levels of antioxidant enzymes viz superoxide dismutase (SOD) and catalase (CAT) and antioxidant reduced glutathione (GSH) in brain and liver. To determine the gender differences at the level of lipid peroxidation, the assessment of MDA levels was also carried out in both the tissues. In addition to this serum lipid profile and liver function tests (LFTs) were assessed to determine the risk of developing atherosclerosis as well as the extent of liver damage respectively in both genders consequent to chronic nicotine exposure in rats. Additionally, any associations between lipid profile versus oxidative stress parameters were also determined.

MATERIALS AND METHODS

Animals

For the experiments, locally raised adult male and female Albino Wistar rats (n=48), weighing 140-170g were bought from International Centre for Chemical and Biological Sciences, University of Karachi and kept in standard lab conditions for three weeks with six rats per cage, including free access to lab chow and water and a 12-hour natural light-dark cycle at 23±2°C. The handling of animals was done in compliance with the National Research Council's (1996) regulations for the use and care of laboratory animals. All methods were used to lessen the number of rats and the suffering they experienced. Ethical approval for the study was taken from the ethics committee for animals, University of Karachi. BASR/No. 03189/Sc.

Drug and treatment

After a one-week acclimation period, rats were randomly separated into 4 groups, including both control and nicotine-treated groups for both male and female (n=12/group). While nicotine-treated groups were given Nicotine Hydrogen Tartrate orally at a dose of 3.08mg/ml/kg body weight/day for 21 days, control groups merely got nicotine-free drinking water. The nicotine dosage was determined using our laboratory's published research (Bibi *et al.*, 2011; Bano and Saeed, 2014). Every day throughout the course of the treatment, body weight was recorded twice.

Collection of samples

After 3.5 hours of last dose on day 21, the rats were euthanized by decapitation, followed by the rapid samples (whole intact perfused livers and brain) isolation on ice and preservation at -70°C until analysis. An isotonic

saline was used to remove the blood from tissues during isolation. Trunk blood from sacrificed rats was bench centrifuged for 15 min at 3000 rpm to get the serum, which was then kept at the temperature of -20°C until use.

Preparation of tissue homogenates

10% homogenate of frozen tissues (liver/brain) of each rat was prepared in 0.1M phosphate buffer (pH~7) on ice with the help of polytron-PT-2100 homogenizer. Using refrigerated centrifuge machine at 4°C the supernatant was obtained, which was then used for the UV/VIS spectrophotometric (model UV 1600 (TOMOS, USA). determination of activities of antioxidant enzymes i.e., CAT and SOD as well as the levels of GSH, albumin and MDA.

Determination of antioxidant status of brain and liver tissues

Estimation of Malondialdehyde (MDA)

The thiobarbituric acid (TBA) reaction was used to determine the amount of MDA in liver and brain tissue homogenates (Uchiyama and Mihara, 1978). Briefly, 3 ml of 1% H₃PO₄ (pH 2) and 1ml of 0.6% TBA solutions were added to 0.5ml of the homogenate of the tissue sample. After stirring, this was placed in a boiling water bath for about 45 min. The mixture was allowed to cool at room temperature and then 4ml of n-butanol was added and vortexed. Bench centrifugation at 3000rpm for 10 minutes was used to separate the butanol (top) layer and its absorbance at 535 and 520nm was measured spectrophotometrically against blank (n-butanol alone). The difference of absorbance was calculated to be taken as the concentration of MDA.

Estimation of catalase (CAT)

A calorimetric method was used to determine the CAT levels in liver and brain tissue supernatants as described previously (Sinha, 1972). To sum-up, the reaction mixture was composed of 0.5ml of tissue supernatant and 0.6ml of H₂O₂ (65mM). After incubation at 37°C for 3min, the reaction was stopped by the addition of 2ml of potassium dichromate/glacial acetic acid (5%, 1:3 by volume). After heating for 10 min and then cooling at room temperature, changes in absorbance were read at 570nm. CAT activity was calculated as µM of H₂O₂ consumed/mg protein.

Estimation of reduced glutathione (GSH)

GSH in tissue supernatants was determined by the Ellman method, using DTNB [5, 5'-dithiobis-(2-nitrobenzoic acid)] as the substrate (Ellman, 1959). 1ml of phosphate buffer (0.1M, pH~7) and 0.25ml of DTNB (5µM) were added to 0.5ml tissue supernatant and then vortexed. The yellow-colored (TNB) developed was read within 15 min at 412nm against reagent blank and expressed as µM of GSH.

Estimation of superoxide dismutase (SOD)

For the quantitative *in vitro* determination of SOD activity in tissues supernatants, commercially available diagnostic

kit (RANSOD) by Randox Labs was used. The method employs superoxide radical's generation by xanthine and xanthine oxidase (XOD). The radicals then form a red-colored formazan dye after reacting with I.N.T (2 - (4 - iodophenyl) - 3 - (4 - nitrophenol) - 5 - phenyltetrazolium chloride). The reaction's degree of inhibition was used to determine the activity of SOD. The increase in absorbance was read spectrophotometrically at 505nm.

Evaluation of biochemical profiles

Determination of LFT

Commercially available ready-to-use diagnostic kits by Merck (Pvt.) Limited were used for the quantitative estimation of enzymatic activities of Alkaline Phosphatase (ALP) and Alanine aminotransferase (ALT) as well as of bilirubin (BR), both total and direct, in the serum samples of rats at 405nm, 340nm and 570nm respectively by using Microlab 300LX clinical analyzer (ELITech). Indirect bilirubin was determined by calculating the difference between total BR and direct BR.

Determination of lipid profile

For the determination of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides in the serum samples of rats, commercially available diagnostic kits by Randox Labs were used. Absorbances were read within 30 min at 500nm against the reagent blank by using UV-VIS spectrophotometer. Concentration of Low-density lipoprotein cholesterol (LDL-C) was obtained with the help of following formula:

$$\text{LDL-C (mg/dl)} = \text{TC} - \text{Triglycerides}/5 - \text{HDL-C}$$

Determination of serum albumin

Serum albumin was determined by Bromocresol green method as described previously (Doumas *et al.*, 1971).

Chemicals

The drug Nicotine Hydrogen Tartrate was acquired from Sigma-Aldrich. All other compounds were of the purest commercially available grades.

STATISTICAL ANALYSIS

The mean and SEM of all data are shown (n=6/group). GraphPad Prism (version 2.01) software was used for the statistical analysis, which involved two-way analysis of variance (ANOVA) and the Newman-Keuls q-test to compare the four groups. P values less than 0.05 were regarded as significant. To evaluate correlations between parameters, Pearson's correlation coefficient was used.

RESULTS

Brain antioxidant status in nicotine-treated male and female rats

Brain CAT, SOD and GSH as well as the lipid peroxidation marker, MDA, are shown in table 1 as indicators of the brain antioxidant state in both male and female rats treated with nicotine. Males were found to have considerably higher MDA levels than the

corresponding controls. However, no significant difference was found in the levels of MDA between nicotine treated females versus their controls. Additionally, a significant drop in brain CAT and SOD levels was found in both nicotine treated male and female rats versus their controls.

GSH amount is increased in females and decreased in male rats. Newman-Keuls q statistics show that in comparison to identically treated male rats, female rats have higher levels of GSH (104.74%, p<0.001), CAT (89.36%, p<0.001) and SOD (65.74%, p<0.001) and less MDA (15.49%, p<0.001) levels.

Liver antioxidant status in nicotine-treated male and female rats

Table 2 shows the liver antioxidant status evaluated by measuring the concentrations of liver CAT, SOD, GSH and MDA in both male and female rats treated with nicotine. In comparison to their respective controls, it was discovered that both male and female rats had significantly lower levels of CAT, SOD and GSH but an increase in MDA concentrations. Comparing female and identically treated male rats, Newman-Keul's q statistics reveals that the levels of CAT (22.42%, p<0.05) SOD (19.83%, p<0.01), GSH (63.73%, p<0.01) are considerably lower whereas MDA levels (111.81%, p<0.01) are significantly higher in the females.

Serum biochemical profiles

LFT profile in Nicotine-Treated Male and Female Rats

Table 3 shows the LFT in nicotine-treated male and female rats, evaluated by measuring the concentrations of ALP, ALT, direct, total as well as indirect BR and albumin in serum. Both female and male rats were shown to have higher levels of ALP, ALT and direct BR while having lower levels of total BR, indirect BR and albumin when compared to the corresponding controls.

According to Newman-Keuls q statistics, female rats had significantly higher levels of ALP (31.33%, p<0.01), ALT (15.50%, p<0.01), direct (25%, p<0.01) and indirect BR (85.71%, p<0.01) than similarly treated male rats.

Lipid profile in nicotine-treated male and female rats

Table 4 shows the lipid profile in nicotine-treated male and female rats, evaluated by measuring the levels of serum TC, HDL-C, LDL-C and triglycerides.

Both male and female rats were found to have increased levels of all the measured parameters than their respective controls with the exception of HDL-C, which was shown to have considerably lower levels.

When comparing female rats to identically treated male rats, Newman-Keuls q statistics showed that the former has considerably higher levels of HDL-C (25.93%, p<0.01) and significantly lower levels of TC (16.58%, p<0.01), LDL-C (30.77%, p<0.01) and triglycerides (8.46%, p<0.01).

Table 1: Brain antioxidant status in nicotine-treated male and female rats

Parameters	Male		Female		Two-way ANOVA (df 1,20)		
	Control	Drug	Control	Drug	Sex	Drug	Sex × Drug
CAT ($\mu\text{M}/\text{mg}$ protein)	7.41 \pm 0.44	3.01 \pm 0.21 **	7.27 \pm 0.26	5.70 \pm 0.23 *†	F=94.42 P<0.01	F=17.22 P<0.01	F=21.36 P<0.01
SOD ($\mu\text{M}/\text{mg}$ protein)	2.64 \pm 0.03	1.08 \pm 0.01 **	2.75 \pm 0.05	1.79 \pm 0.02 **†	F=599.13 P<0.01	F= 63.09 P<0.01	F=33.67 P<0.01
GSH ($\mu\text{M}/\text{mg}$ protein)	4.30 \pm 0.05	2.32 \pm 0.06 **	4.18 \pm 0.04	4.75 \pm 0.07 **†	F=127.18 P<0.01	F=340.39 P<0.01	F=413.82 P<0.01
MDA (nM/mg protein)	1.55 \pm 0.07	2.13 \pm 0.02 **	1.43 \pm 0.03	1.80 \pm 0.04 †	F=102.42 P<0.01	F=23.33 P<0.01	F=4.93 P<0.05

Experimental details are given in the materials and methods section. All values presented as means \pm SEM for each group of 6 rats. Statistical analysis was performed using two-way ANOVA followed by Newman Keul's q-test. The significance of differences is indicated by *p<0.01, **p<0.001 when drug treated group was compared with respective control group and †p<0.001 when female groups were compared with their male counterparts. CAT: catalase; SOD: superoxide dismutase; GSH, reduced glutathione; MDA, malondialdehyde.

Table 2: Liver antioxidant status in nicotine-treated male and female rats

Parameters	Male		Female		Two-way ANOVA (df 1,20)		
	Control	Drug	Control	Drug	Sex	Drug	Sex × Drug
CAT ($\mu\text{M}/\text{mg}$ protein)	7.02 \pm 0.32	4.46 \pm 0.28 *	7.07 \pm 0.23	3.46 \pm 0.24 *†	F=123.62 P<0.01	F=2.88 NS	F=3.52 NS
SOD ($\mu\text{M}/\text{mg}$ protein)	1.84 \pm 0.02	1.21 \pm 0.01 *	1.95 \pm 0.04	0.97 \pm 0.05 *†	F=343.48 P<0.01	F= 1.76 NS	F=11.16 P<0.01
GSH ($\mu\text{M}/\text{mg}$ protein)	4.33 \pm 0.11	2.84 \pm 0.17 *	4.09 \pm 0.09	1.03 \pm 0.068 *†	F=329.15 P<0.01	F=66.18 P<0.01	F=39.30 P<0.01
MDA (nM/mg protein)	0.95 \pm 0.08	1.27 \pm 0.05**	1.14 \pm 0.12	2.69 \pm 0.32 *††	F=26.24 P<0.01	F=19.37 P<0.01	F=11.79 P<0.01

Experimental details are given in the materials and methods section. All values presented as means \pm SEM for each group of 6 rats. Statistical analysis was performed using two-way ANOVA followed by Newman Keul's q-test. The significance of differences is indicated by *p<0.01 and **p<0.001 when drug treated group was compared with respective control group and †p<0.05, ††p<0.01, when female groups were compared with their male counterparts. CAT: catalase; SOD: superoxide dismutase; GSH, reduced glutathione; MDA, malondialdehyde.

Table 3: Serum LFT in nicotine-treated male and female rats

Parameters	Male		Female		Two-way ANOVA (df 1,20)		
	Control	Drug	Control	Drug	Sex	Drug	Sex × Drug
ALP (U/L)	42.29 \pm 1.27	75.24 \pm 2.68 *	39.87 \pm 1.76	98.82 \pm 2.59 *†	F=454.00 P<0.01	F=24.03 P<0.01	F=36.34 P<0.01
ALT (U/L)	22.50 \pm 0.76	64.50 \pm 3.33 *	21.70 \pm 0.88	74.50 \pm 2.39 *†	F=494.28 P<0.01	F= 4.61 P<0.05	F=6.45 P<0.05
Direct BR (mg/dl)	0.16 \pm 0.01	0.24 \pm 0.01 *	0.19 \pm 0.01	0.30 \pm 0.01 *†	F=12.92 P<0.01	F=56.32 P<0.01	F=0.67 NS
Total BR (mg/dl)	1.02 \pm 0.07	0.62 \pm 0.04 *	1.14 \pm 0.01	0.51 \pm 0.06 *	F=153.82 P<0.01	F=2.59 NS	F=8.22 P<0.01
Indirect BR (mg/dl)	0.66 \pm 0.03	0.21 \pm 0.01 *	0.70 \pm 0.04	0.39 \pm 0.02 *†	F=137.67 P<0.01	F=10.90 P<0.01	F=4.77 P<0.05
Albumin (gm/dl)	9.11 \pm 2.41	7.29 \pm 1.64 *	9.16 \pm 2.10	6.39 \pm 1.58 *	F=1.36 NS	F=37.37 P<0.01	F=1.52 NS

Experimental details are given in the materials and methods section. All values presented as means \pm SEM for each group of 6 rats. Statistical analysis was performed using two-way ANOVA followed by Newman Keul's q-test. The significance of differences is indicated by *p<0.01 when drug treated group was compared with respective control group and †p<0.01 when female groups were compared with their male counterparts. LFT, liver function test; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BR, bilirubin.

Table 4: Serum lipid profile in nicotine-treated male and female rats

Parameters	Male Control Drug		Female Control Drug		Two-way ANOVA (df 1,20) Sex Drug Sex × Drug
TC (mg/dl)	140.30 ± 7.90	231.20 ± 2.85 *	147.30 ± 1.71	192.85 ± 3.30 *††	F=218.31 F=11.55 F=24.09 P<0.01 P<0.01 P<0.01
HDL-C (mg/dl)	45.83 ± 1.50	32.00 ± 1.40 *	57.06 ± 2.31†	40.30 ± 1.29 *††	F=33.70 F= 82.71 F=0.76 P<0.01 P<0.01 NS
LDL-C (mg/dl)	70.14 ± 4.90	162.00 ± 3.06 *	71.68 ± 2.64	112.14 ± 3.00 *††	F=351.58 F=47.15 F=53.32 P<0.01 P<0.01 P<0.01
Triglycerides (mg/dl)	121.70 ± 3.50	202.06 ± 2.61 *	124.80 ± 2.60	184.95 ± 3.90 *††	F=470.66 F=4.75 F=9.66 P<0.01 P<0.05 P<0.01

Experimental details are given in the materials and methods section. All values presented as means ± SEM for each group of 6 rats. Statistical analysis was performed using two-way ANOVA followed by Newman Keul's q-test. The significance of differences is indicated by *p<0.01 when drug treated group was compared with respective control group and †p<0.05, ††p<0.01 when female groups were compared with their male counterparts. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Table 5: Pearson's correlation coefficient r value between parameters of oxidative stress and antioxidant status versus lipid profile as well as ALT and serum bilirubin in nicotine treated male rats

	Liver SOD	Liver Catalase	Liver GSH	Brain SOD	Brain Catalase	Brain GSH	Brain MDA
Total Cholesterol	0.045	-0.591	-0.545	0.148	0.106	-0.434	0.054
LDL-C	0.147	-0.597	-0.575	0.233	0.287	-0.458	0.277
HDL-C	-0.036	0.419	0.652	-0.180	-0.549	0.617	-0.892*
Triglycerides	-0.346	-0.550	-0.881*	-0.053	0.234	-0.871*	0.684
ALT	-0.148	-0.453	-0.606	0.382	0.616	-0.504	0.512
Serum Bilirubin	0.376	-0.509	-0.440	0.236	0.697	0-076	0.429

Table 6: Pearson's correlation coefficient r value between parameters of oxidative stress and antioxidant status versus lipid profile as well as ALT and serum bilirubin in nicotine treated female rats.

	Liver SOD	Liver Catalase	Liver GSH	Brain SOD	Brain Catalase	Brain GSH	Brain MDA
Total Cholesterol	-0.457	-0.192	-0.386	0.618	-0.227	0.337	0.078
LDL-C	-0.445	-0.036	-0.340	0.362	-0.307	0.253	0.280
HDL-C	-0.337	-0.358	-0.207	0.745	0.038	0.362	-0.270
Triglycerides	0.474	-0.131	0.004	0.022	0.217	-0.200	-0.441
ALT	-0.798	-0.549	0.599	-0.259	0.230	-0.554	-0.019
Serum Bilirubin	-0.299	-0.349	-0.787	0.780	-0.497	0.274	0.038

*Correlation is significant at the 0.05 level. SOD: superoxide dismutase; GSH, reduced glutathione; MDA, malondialdehyde; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ALT, alanine aminotransferase.

Correlations between lipid profile parameters and LFTs versus parameters of oxidative stress and antioxidant status in nicotine treated male (table 5) and female (table 6) rats.

Serum triglycerides were found to be significantly inversely correlated to both liver and brain GSH in nicotine treated male rats (fig. 1 and 2 respectively). Also, serum HDL was also found to have a significant inverse relationship with brain MDA in nicotine treated male rats (fig. 3).

However, no significant correlations were determined between lipid profile parameters and LFTs versus parameters of oxidative stress and antioxidant status in nicotine treated female rats (table 6).

DISCUSSION

The objective of the current study was to evaluate the gender-specific effects of chronic nicotine administration on oxidative stress and antioxidant status measures in rat brain and liver. In addition to this, LFTs were measured to assess the gender-based differences in the extent of liver damage as well as gender-based differences in the lipid profile were also assessed.

The study showed that prolonged nicotine use elevated MDA levels and lowered SOD and CAT activity in both sexes' liver and brain tissues. However, in females, GSH was found to be higher in brain and lower in liver than their male counterparts. Concentrations of ALP, ALT, direct and indirect bilirubin were found to be greater in females. Moreover, disturbances in lipid profile were less pronounced in female than male rats.

Our finding of increased MDA levels in liver of both sexes agrees to a non-gender-based study that showed raised levels of lipid peroxidation products in the liver on chronic exposure to nicotine (Baskaran *et al.*, 2000). However, the latter study revealed no change in the brain tissue as compared to the controls which is in contradiction to our findings. Additionally, the liver and brain tissues of both sexes exhibit reduced SOD and CAT activity in the current study, however, GSH was found to be higher in brain and lower in liver of females as compared to those of their male counterparts. In contrast to this, another study that used Ferric Reducing Antioxidant Power (FRAP) and 2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) assays reported higher antioxidant capacity in the liver in females than male rats (Katalinic *et al.*, 2005). Another study that revealed significantly raised MDA levels and decreased activities of super oxide dismutase as well as reduced glutathione in both sexes depicted that female are less susceptible to nicotine toxicity to the liver tissue than males (Zahran and Osman, 2008). Another study has also shown increased MDA levels and decrease in the levels of total antioxidant status as well as Glutathione peroxidase and SOD in the brain consequent to exposure to nicotine (Biala *et al.*, 2017). However, we could not find any study depicting the gender-specific effects of chronic nicotine administration on oxidative stress in the brain.

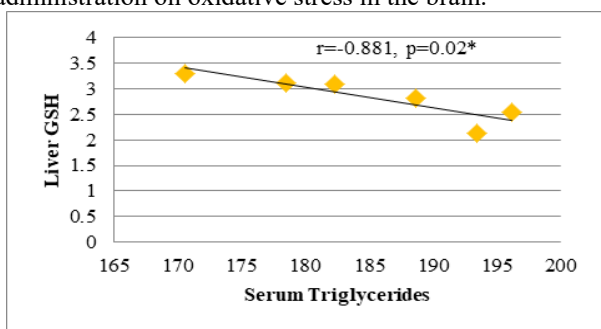


Fig. 1: Negative linear correlation between Serum triglycerides and liver GSH in nicotine treated male rats. GSH= Reduced glutathione. *Correlation is significant at the 0.05 level.

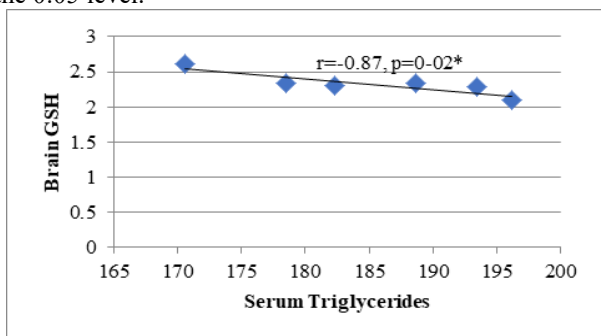


Fig. 2: Negative linear correlation between serum triglycerides and brain GSH in nicotine treated male rats. GSH= Reduced glutathione. *Correlation is significant at the 0.05 level.

It has been proposed that nicotine administration could lead to alterations in serum lipid profile through the release of catecholamines, cortisol and growth hormones that in turn causes lipolysis of stored triglycerides in adipose tissue with release of free fatty acids ultimately resulting in enhanced levels of TG and VLDL (Jain and Ducatman, 2018). Nicotine-induced boosts in plasma free fatty acids deposition is also the source of polyunsaturated fatty acids for the brain where their high concentration act as the substrates for ROS (Biala *et al.*, 2017), demonstrated by the greater nicotine-induced oxidative stress in brain tissue of males than female rats in our study.

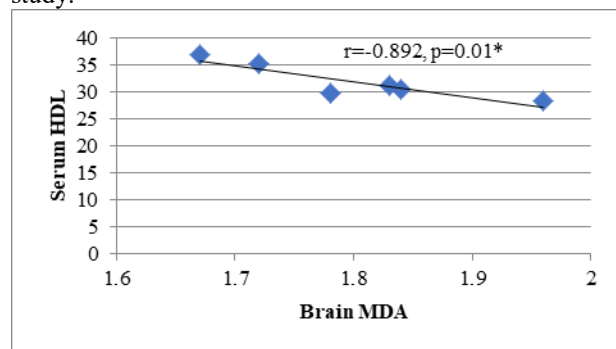


Fig. 3: Negative linear correlation between Serum HDL and brain MDA in nicotine treated male rats. HDL=High density lipoprotein, MDA=Malondialdehyde. *Correlation is significant at the 0.05 level.

Assessment of serum lipids in the present study demonstrated an atherosclerotic profile with an elevation in TC, LDL-C and triglycerides levels along with a significant reduction in HDL-C level upon prolonged nicotine administration in both male and female rats (table 4). It is generally believed that sex hormones control plasma lipid levels, causing the lipid profile to be sexually dimorphic (Žitňanová *et al.*, 2018), as shown by the difference in HDL-C levels in control groups of both sexes in the current study. Our findings unveil a potential relationship between chronic nicotine use and dyslipidemia in both genders with greater dyslipidemic effects in male rats. Our results are in line with earlier research on mice conducted in our lab (Bibi *et al.*, 2011; Bano and Saeed 2014). Additionally, another study that compared female smokers to male smokers found that female smokers had greater levels of HDL and lower levels of TG and LDL. However, in contradiction to the findings in our study, enhanced levels of TC in females as compared to male smokers were reported in that study (Jain and Ducatman, 2018).

Nicotine-induced lipid peroxidation is associated with the pathogenesis of atherosclerosis and plays a central role in the initiation and progression of CVDs in smokers (Akbarbartoori *et al.*, 2006). Comparatively higher levels of HDL-C with lower levels of TC, LDL-C and triglycerides in females than similarly treated male rats

indicates sex-based differences and suggests a less disturbed lipid profile in females upon nicotine intake than males, making them less prone to cardiovascular episodes. This may be because of the presence of estrogen in females, which have a tremendous importance in the pathophysiology of cardiovascular diseases (Dessalvi *et al.*, 2019). The protective effect of high levels of estrogens throughout a woman's reproductive life has been linked to the lower rate of CVDs in females than males (do Nascimento *et al.*, 2019).

The findings of increased levels of serum ALP, ALT and direct BR with decreased concentrations of total as well as indirect BR and albumin in both nicotine-treated male and female rats (table 3) indicates the destructive effects of chronic nicotine administration on liver in both sexes. The results of the current study are consistent with those of other studies also that have shown raised levels of liver enzymes (ALP and ALT) in serum upon nicotine administration at a dose of 2.5mg/kg in mice (Salahshoor *et al.*, 2016) and in smokers (Kester and Alfred, 2018). Increased level of liver cytosolic enzymes in blood may be due to the damage of liver cell membrane or necrosis due to nicotine (Salahshoor *et al.*, 2019). It appears that nicotine-induced free radicals production triggers the lipid peroxidation thus causing cell damage (Cordova *et al.*, 2002). Kester and Alfred linked the increased levels of liver enzymes with increased oxidative stress in cigarette smokers (Kester and Alfred, 2018) which is also confirmed by our findings. Moreover, this increase in ALP, ALT along with direct-BR levels and decrease in indirect-BR level is greater in female rats as compared to similarly treated male rats indicating a more pronounced damaging effect of chronic nicotine on liver functions in females. This could be due to the manifestation of greater oxidative processes in female liver as compared to male found in the present study. In contrast to our findings, a study conducted to assess hepatic antioxidant status as well as the histopathological changes consequent to administration of nicotine, has shown that effects of nicotine are more pronounced in males as compared to females (Zahran and Osman, 2008).

Significant negative correlations between serum triglycerides versus brain and liver GSH as well as between brain MDA versus HDL in nicotine treated male rats indicate that increase in the levels of triglycerides and decreased HDL are manifestations of oxidative stress. However, no such relationships were found in nicotine treated female rats that again point towards the protective effects of estrogen against derangements in lipid profile.

CONCLUSION

Thus, it is concluded that owing to the lowered GSH in liver in females compared to males, the former suffered more from oxidative stress related damage to the liver cells as indicated by their increased levels of ALP, ALT

and bilirubin. Reduced oxidative stress in the brain cells of females as compared to males may act as a protective measure against the onset of neurodegenerative illnesses. Also, the lesser derangements in lipid profile in females compared to males point toward the protective effects of estrogen in the former. However, raised triglycerides and decreased HDL might be considered as manifestations of oxidative stress in nicotine treated male rats as evident by significant negative correlations of these parameters with brain and liver GSH and brain MDA.

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