Original Article

Chronic Effects of Nicotine on Serum Lipid Profile and Oxidative Stress: An Experimental Study

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ABSTRACT

Objective: To examine the chronic effect of nicotine on serum lipid profile and oxidative stress in male mice.
Study Design: An Experimental Animal Study
Place and Duration of Study: This study was conducted at the Department of Biochemistry, Liaquat College of Medicine & Dentistry, Karachi from February 2020 to April 2020 for a period of two months.
Materials and Methods: Adult male BALB/C mice weighing 25-30 gm were housed in six cages under light and dark settings at 25ºC and fed lab chow and water ad libitum under regular housing circumstances. Two sets of mice were created (control and test). There were 12 mice in each group. The drug-treated group received nicotine hydrogen tartrate (3.08 mg/ml) in 100 mL of drinking water for 28 days, whereas the control group got tap water.
Results: After chronic nicotine delivery, there was a significant difference in glucose concentration, body weight, and plasma albumin concentration (P<0.01). Between the control and test groups, there was a significant difference in LDL-C and triglycerides (P<0.001). The levels of total cholesterol and HDL-C are unaltered. MDA levels in the liver (P<0.001), reduced glutathione and catalase activity (P<0.01), and brain MDA, reduced glutathione and catalase activity (P<0.01), (P<<0.001), and (P0.001), respectively, were all determined to be significant.
Conclusion: In conclusion chronic administration of nicotine caused change in lipid profile, promoted lipid peroxidation and significantly reduced liver antioxidant enzyme activities in mice. It could be inferred that nicotine users have increased LDL-C and triglycerides which makes them more vulnerable to cardiovascular events.
Key Words: Serum Lipid Profile, Oxidative Stress, Chronic, Tobacco

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INTRODUCTION

Smoking is one of the most important risk factors for the development of coronary atherosclerosis and coronary heart disease. Smoking, which is a known risk factor for the development of ischemic heart disease, can cause a change in the normal plasma lipoprotein pattern.[1] Elevated Total lipid levels are thought to play a role in the progression of atherosclerosis.[2]

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Triglyceride levels were also shown to be greater in smokers than in non-smokers. According to recent research, triglyceride levels are the most major element in the development of CHD.[3] Nicotine is one of the most common chemicals found in tobacco smoke. Nicotine raises the levels of triglycerides, cholesterol, and VLDL while lowering the levels of HDL. Cluette Brown found that Long-term oral nicotine intake was also found to raise LDL cholesterol while lowering HDL cholesterol.[4] Nicotine has been demonstrated to increase the circulatory pool of atherogenic LDL by speeding up the transfer of lipids from HDL and hindering LDL clearance from the plasma compartment, resulting in more LDL cholesterol deposition in the arterial wall.[5]

An oxidative stress is defined as a lack of equilibrium between reactive oxygen species (ROS) production and antioxidant defenses, which results in free radicals and, as a result, malondialdehyde (MDA) formation due to membrane lipid peroxidation.[6,7] Nicotine administration has been shown to reduce endogenous antioxidant status, such as superoxide dismutase (SOD) and glutathione peroxidase (GPX) activity.[8]

Previous study has shown that smoking reduces insulin sensitivity, promotes insulin resistance, and increases cardiovascular risk factors such greater plasma triglycerides, decreased HDL-C, and hyperglycemia. A

lot of studies have connected smoking to metabolic abnormalities and metabolic syndrome.[9-11]

Chronic nicotine treatment in forced swim mice fails to alleviate depression-like symptoms, worsens lipid profiles, and impairs glucose homeostasis, which could be linked to a higher risk of cardiovascular disease and depression in chronic smokers, particularly those who live in stressful situations.[12] This experimental study was designed to examine the chronic effect of nicotine on serum lipid profile and oxidative stress in male mice.

MATERIALS AND METHODS

The following animal procedures were carried out in strict accordance with the standards for the care and use of laboratory animals published by the National Research Council (1996). The institutional animal ethics committee at the University of Karachi provided their approval. All efforts were taken to keep the number of animals as low as possible, as well as any pain or anguish they might experience. Adult male BALB/C mice weighing 25-30 gm were kept in six cages at 25ºC, with light and dark settings, and fed lab food and water ad libitum under normal living conditions. There were two sets of mice developed (control and test). Each group consisted of 12 mice. The drug-treated group received nicotine hydrogen tartrate (3.08 mg/ml) in 100 mL of drinking water for 28 days, whereas the control group received tap water. Animals were decapitated after the last treatment. Blood was drawn and centrifuged for 30 minutes at 4000 rpm. The serum was separated as a supernatant and kept at –20°C until further analysis.

Serum Parameters Analysis the O-toluidine technique was used to determine serum glucose concentrations. [13] The dye-binding technique was used to assess serum albumin concentrations. [14] Kit technique was used to calculate serum cholesterol, triglycerides, HDL-C, and LDL-C (Randox®, Private Ltd). Determination of serum Protein by Lowry's Method (1951). Colorimetric Assay of Catalase by Sinha et al., 1972, Estimation of Reduced Glutathione by Ellman (1959) and Determination of Malonaldehyde with Thiobarbituric Acid Test by Uchiyama and Mihara (1977). Drugs and Chemicals Sigma Chemical Co. provided Nicotine Hydrogen (+)-tartrate. The rest of the compounds were of analytical quality.

All data are expressed as the mean minus the standard error of the mean. A two-tailed student t-test was used to determine the significance of the difference between the comparing means. If the accompanying P (probability of error) values were less than 0.05, all values were considered statistically significant.

RESULTS

The effects of nicotine on glucose concentration, body weight, and plasma albumin concentration in mice are shown in Figure 1. After chronic nicotine delivery, there was a significant difference in glucose concentration, body weight, and plasma albumin concentration ($P<0.01$).

The effects of chronic nicotine delivery on the lipid profile in mice are shown in Table 1. Between the control and test groups, there was a significant difference in LDL-C and triglycerides ($P<0.001$). The levels of total cholesterol and HDL-C are unaltered.

Table 2 shows the effects of chronic administration of nicotine on liver oxidative stress parameters in mice. A significant difference was found between MDA levels ($P<0.001$), reduced glutathione and catalase activity ($P<0.01$). Table 2 reveals the impact of prolonged nicotine delivery on the oxidative stress status of mice's brains. MDA, reduced glutathione, and catalase activity were all shown to be substantially different ($P<0.01$, $P<0.001$), ($P<0.001$), and ($P<0.001$), respectively.

Table No.1: Effects of chronic nicotine administration on plasma lipid Profile

Parameter	Control mean ± SEM	Test mean ±SEM	p-value
Total Cholesterol (mg/dl)	163.9±13.01	192.3±14.4	>0.05
HDL-Cholesterol (mg/dl)	36.5±2.95	32.52±1.58	>0.05
LDL-Cholesterol (mg/dl)	49.2±4.43	66.8±3.01	<0.001
Triglycerides (mg/dl)	70.6±2.26	85.5±3.53	<0.001

Table no.2: Effect of Chronic Nicotine Administration on Liver & Brain Antioxidant Status

Parameter		Control mean ± SEM	Test mean ± SEM	p-value
Liver	MDA (nM/g of tissue)	22.4±0.25	25.8±0.46	<0.001
	Reduced Glutathione (µM/g of tissue)	6.3±0.21	4.4±0.88	<0.01
	Catalase (µM/min/mg of protein)	0.1±0.00	0.07±0.00	<0.01
Brain	MDA (nM/g of tissue)	15.8±0.28	18.1±0.65	<0.01
	Reduced Glutathione (µM/g of tissue)	2.3±0.2	5.08±0.47	<0.001
	Catalase (µM/min/mg of protein)	0.13±0.003	0.2±0.01	<0.001

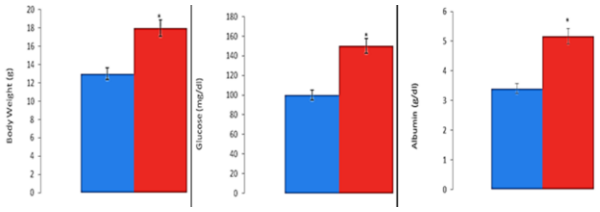

Figure No.1: Effects of Chronic Nicotine Administration on Plasma Glucose Concentration, Body Weight and Plasma Albumin Concentration

DISCUSSION

The current study reveals that giving mice nicotine (3.08 mg/kg/day) for four weeks boosts their body weight and glucose levels. Previous studies suggested that smoking itself may triggers metabolic syndrome by increasing body weight and glucose concentration. Increased glucose concentration causes more glucose to reach pancreatic beta cells, whereas increased glycolysis speeds up insulin secretion. Smoking is likely to promote insulin resistance directly. [15] As a result, smoking raises the risk of metabolic syndrome and diabetes, which, in turn, raises the risk of cardiovascular disease.

Our results indicate that chronic nicotine treatment increases plasma albumin concentration. Recent studies in contrast with our results showed that Plasma albumins are lower in smokers because albumin has an antioxidant characteristic that can be observed by increased oxidative stress.[16,17] Nicotine therapy also elevated triglycerides and LDL-C levels in mice. Serum HDL-C, on the other hand, remained unchanged. These findings are consistent with Bibi et al earlier findings (2011).[18] Our results is consistent with previous studies that cigarette leads to increase in the concentration of serum total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol and fall in the levels of anti atherogenic HDL- cholesterol, as reported by Muscat.[19] Nicotine stimulates adrenaline secretion, which causes glucose to be released from the liver into the bloodstream. We found MDA levels were increased while reduced glutathione and catalase levels were decreased. Our findings are in line with research that show elevated MDA levels cause lipid peroxidation.[20] In nicotine-treated rats, increased lipid peroxidation products are linked to poorer activity of cleaning enzymes such as glutathione and catalase.[21]

In our study brain MDA levels, reduced glutathione and catalase activities were increased in nicotine administered mice. In contrast to our findings, Baskaran et al. found no significant difference in brain MDA levels across rats, implying that nicotine did not promote free radical-mediated tissue damage in the hippocampus. However, our findings are in line with prior research, which found that when 3mg/kg of nicotine was administered, the level of reduced glutathione increased. Furthermore, CAT levels rose considerably.[22] Nicotine administration, according to some, can cause oxidative stress by causing the production of reactive oxygen species in the peripheral and central nervous systems.[23] Furthermore, it has been suggested that nicotine at extremely low doses may function as an antioxidant and play an essential part in its neuroprotective action, but a high dose of nicotine may cause neurotoxicity and induce oxidative stress[24].

CONCLUSION

In mice, chronic nicotine administration changed their lipid profile, accelerated lipid peroxidation, and dramatically lowered liver antioxidant enzyme activity, according to this study. Nicotine smokers are thought to have higher LDL-C and triglycerides, making them more sensitive to cardiovascular problems. Therefore, by quitting smoking and regulating metabolic risk variables reduces the chance of significant adverse cardiovascular events.

Author's Contribution:

Concept & Design of Study:	Shabana Saeed, Muhammad Azhar Hussain, Muhammad Athar Khan
Drafting:	Zunairah Rais, Kamal Ahmad
Data Analysis:	Muhammad Athar Khan, Tabinda Ashfaq
Revisiting Critically:	Zunairah Rais, Kamal Ahmad, Tabinda Ashfaq
Final Approval of version:	Shabana Saeed, Muhammad Azhar Hussain, Muhammad Athar Khan

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Stallones RA. The association between tobacco smoking and coronary heart disease. Int J Epidemiol 2015;44(3):735-43.
2. Linton MRF, Yancey PG, Davies SS, Jerome WG, Linton EF, Song WL, et al. The Role of Lipids and Lipoproteins in Atherosclerosis. [Updated 2019 Jan 3]. In: Feingold KR, Anawalt B, Boyce A, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK343489/
3. Upadhyay RK. Emerging risk biomarkers in cardiovascular diseases and disorders. J Lipids 2015;2015:971453.
4. Rao Ch S, Subash YE. The effect of chronic tobacco smoking and chewing on the lipid profile. J Clin Diagn Res 2013;7(1):31-4.

5. Hojnack JM, Cluette-Brown J. Oral nicotine impairs clearance of plasma Low Density Lipoproteins. Proc Soc Exp Biol Med 1986:182;414-18.
6. Betteridge DJ. What is oxidative stress? Metabolism 2000;49(2 Suppl 1):3-8.
7. Srinivasan KN, Pugalendi K. Effect of excessive intake of thermally oxidized sesam oil on lipids, lipid peroxidation and antioxidants status in rats. Ind J Exp Biol 2000:38;777-80.
8. Agarwal P, Bagewadi A, Keluskar V, Vinuth DP. Superoxide dismutase, glutathione peroxidase, and catalase antioxidant enzymes in chronic tobacco smokers and chewers: A case-control study. Ind J Dent Res 2019;30(2):219-225.
9. Benowitz NL. Cigarette smoking and cardiovascular disease: pathophysiology and implications for treatment. Progress in Cardiovascular Diseases 2003;46(1):91–111.
10. Thelle DS, Sharper AG. Blood lipids in middle-aged Britishmen. Br Heart J 1983: 49;205-13.
11. Kim SW, Kim HJ, Min K, Lee H, Lee SH, Kim S, et al. The relationship between smoking cigarettes and metabolic syndrome: A cross-sectional study with non-single residents of Seoul under 40 years old. PLoS One 2021;16(8):e0256257.
12. Facchini FS, Hollenbeck CB, Jeppesen J, Chen YD, Reaven GM. Insulin resistance and cigarette smoking. Lancet 1992; 339:1128-30.
13. Targher G, Alberiche M, Zenere MB, Bonadonna RC, Muggeo M, Bonora E. Cigarette smoking and insulin resistance in patients with noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 1997;82(11): 3619-24.
14. Dumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. 1971. Clin Chim Acta 1997;258(1):21-30.
15. Artese A, Stamford BA, Moffatt RJ. Cigarette Smoking: An Accessory to the Development of Insulin Resistance. Am J Lifestyle Med 2017;13(6):602-605.
16. Roohi N, Mehjabeen, Ashra S. Effect of cigarette smoking on serum proteins profile in male active and passive smokers. Punjab Univ J Zool 2017;32(2): 209-215.
17. Shaika FB, Nagajothi G, Swarnalatha K, Kumar CS, Rajendra W, Maddu N. Correlation between smokeless tobacco (Gutkha) and biomarkers of oxidative stress in plasma with cardiovascular effects. Heliyon 6 2020;e05487.
18. Bibi Z, Saeed S and Bano S. Metabolic and Behavioral Effects of Nicotine in Swim Stressed Mice. J Pharm Nutr Sciences 2011;1:54-60.
19. Muscat JE, Harris RE, Haley NJ, Wynder EL. Cigarette smoking and plasma cholesterol. Am Heart J. 1991;121(1 Pt 1):141-7.
20. Suleyman H, Gumustekin K, Taysi S, Keles S, Oztasan N, Aktas O, et al. Beneficial effects of Hippophae rhamnoides L. on nicotine induced oxidative stress in rat blood compared with vitamin E. Biol Pharm Bull 2002;25(9):1133-6.
21. Helen A, Krishnakumar K, Vijayammal PL, Augusti KT. Antioxidant effect of onion oil (Allium cepa. Linn) on the damages induced by nicotine in rats as compared to alpha-tocopherol. Toxicol Lett 2000;116(1-2):61-8.
22. Jain A, Flora SJ. Dose related effects of nicotine on oxidative injury in young, adult and old rats. J Environ Biol 2012;33(2):233-8.
23. Qiao D, Seidler F, Slotkin T. Oxidative mechanisms contributing to the developmental neurotoxicity of nicotine and chlorpyrifos. Toxicol Appl Pharmacol 2005;206:17–26.
24. Guan ZZ, Yu WF, Nordberg A. Dual effects of nicotine on oxidative stress and neuroprotection in PC12 cells. Neurochem Int 2003;43(3):243-9.