Effect of nicotine on lipid profile, peroxidation & antioxidant enzymes in female rats with restricted dietary protein

K. Chattopadhyay & B.D. Chattopadhyay*

Department of Physiology, University of Calcutta & *Department of Physics, Jadavpur University
Kolkata, India

Received February 28, 2007

Background & objectives: Nicotine intake through tobacco is very common in female population of lower socioeconomic level who are deprived of healthy diet. Women suffer consequences of smoking such as cardiovascular disorder, lung related diseases and oxidative stress, etc. No data are available of the influences of nicotine on lipid profile, lipid peroxidation and antioxidant enzymes levels under restricted dietary protein intake. The present study was carried out to investigate the effect of nicotine on such parameters of female rats fed with protein restricted diet (5% casein) as compared to those with normal protein diet (18% casein) with or without vitamin C or E supplementation.

Methods: Subcutaneous injections of nicotine tartrate (3.5 mg/kg body weights per day for 15 days) were given to the rats and subsequent measurements of plasma lipid profile, plasma and ovary lipid peroxidation and antioxidant enzymes were done.

Results: The results showed significant ($P<0.01$) increase of total cholesterol (TC) and more significant ($P<0.001$) increase of triglyceride and low-density lipoprotein cholesterol (LDL-C) of plasma under both dietary conditions. The increase of plasma very low-density lipoprotein cholesterol (VLDL-C) was highly significant under protein-restricted diet. The high-density lipoprotein cholesterol (HDL-C) decreased significantly in both dietary conditions. Lipid peroxidation in plasma increased significantly in protein-restricted condition. Superoxide dismutase and catalase activities in the ovary tissue decreased significantly ($P<0.001$) by nicotine treatment in both dietary groups.

Interpretation & conclusions: Our findings indicated that nicotine-induced toxicity is more in lipid profile (plasma) and lipid peroxidation (plasma and ovary tissue) under protein-restricted diet as compared to that of the normal protein diet. The antioxidant vitamins antagonized the nicotine-induced effects less effectively on the observed parameters under restricted dietary protein.

Key words Antioxidant enzymes - diet - lipid peroxidation - lipid profile - nicotine
cardiovascular disorders\textsuperscript{2,3}, pulmonary diseases\textsuperscript{4} and oxidant stress\textsuperscript{5}. Short-term experiments with humans have shown that oral administration of nicotine raises plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), and lowers high-density lipoprotein cholesterol (HDL-C) in normal dietary condition\textsuperscript{6}. These observations are clinically important because of the widespread use of smokeless tobacco products such as nicotine containing chewing gum\textsuperscript{7} and nicotine lozenge\textsuperscript{8} in smoking cessation therapy.

Nicotine also induces oxidative stress both \textit{in vivo} and \textit{in vitro} that causes a peroxidant/antioxidant imbalance in blood cells, blood plasma and tissues\textsuperscript{9}. Oxidative stress generates free radicals that attack on the membrane lipids resulting in the formation of malondialdehyde (MDA), which causes peroxidative tissue damage\textsuperscript{10}. Animals studies have shown significantly higher liver and serum levels of MDA, conjugated dienes, hydroperoxides, and free fatty acids in rats induced by cigarette smoke\textsuperscript{11,12}.

Smokers incur a sustained free radical load that increases their ascorbic acid (vitamin C)\textsuperscript{13} and \textgreek{a}-tocopherol (vitamin E)\textsuperscript{14} requirement. Supplementation with ascorbic acid and \textgreek{a}-tocopherol is considered safe and easy, because these are susceptible to dietary manipulation\textsuperscript{15}.

No detailed studies on the influences of nicotine on lipid profile, lipid peroxidation and ovarian antioxidant enzymes have been performed under restricted dietary in take. We have earlier shown nicotine-induced alterations on reproductive organs of female rats under protein-restricted condition and that the degree of nicotine toxicity increased with dietary protein inadequacy\textsuperscript{16}. This study was carried out to investigate the effect of nicotine on plasma lipid profile, lipid peroxidation in plasma and ovary and ovarian antioxidant enzymes of Wistar strain female albino rats both under normal protein and restricted dietary protein conditions, with or without supplementation of vitamin C and E.

**Material & Methods**

**Chemicals:** Nicotine tartrate and other fine chemicals were purchased from Sigma Chemical Company, USA. Salt and vitamin mixtures were purchased from Merck, Germany. All other chemicals and reagents were purchased from Sisco Research Laboratory Pvt Ltd (SRL), India, and were of analytical grade. Biochemical reagent kits was supplied by Ranbaxy Diagnostic Limited, Mumbai, India.

**Animals and diet:** Adult female albino rats (n=80) of Wistar strain of body weight 120-130 g were procured from the animal housing facility, Department of Physiology, University of Calcutta, Kolkata. They were maintained in accordance with the guidelines of the rule of Institutional Animal Ethics Committee of Jadavpur University, Kolkata, and were housed in polypropylene cages and fed standard pellet diet (Hindusthan Lever Ltd, India) for 1 wk and water \textit{ad libitum}. Animals were divided into two groups of 40 animals each. Each group was further divided into four subgroups consisting of 10 animals each as follows:

- **Subgroup 1:** Control (without nicotine treatment)
- **Subgroup 2:** Nicotine treated
- **Subgroup 3:** Nicotine treated with vitamin C supplemented in diet
- **Subgroup 4:** Nicotine treated with vitamin E supplemented in diet.

One group was maintained with normal protein diet (18% casein, 70% amyllum, 7% fat, 4% salt mixture and 1% vitamin mixture) and the other group with protein restricted diet (5% casein, 83% amyllum, 7% fat, 4% salt mixture and 1% vitamin mixture) according to Hawk \textit{et al}\textsuperscript{17}. In vitamin C or vitamin E supplemented dietary mixture, 1000 mg of vitamin C or1600 mg of vitamin E was mixed respectively per kg normal/protein restricted diet\textsuperscript{18,19}. Animals were pair-fed for 4 wk in their respective dietary regimen before starting the treatment. The dietary status for each group was maintained till the completion of nicotine treatment.

**Mode of treatment:** The dose and the administration route of nicotine were based on our earlier study\textsuperscript{16}. Animals in all treatment subgroups (2,3 and 4) of both group received subcutaneous injection of nicotine tartrate (dissolved in 0.9% physiological saline) at an effective dose of 3.5 mg/ kg body weights daily for 15 days at 1600 h every day to avoid diurnal variation. The dilution was done in such a way that 1 ml of physiological saline contains the required dose of nicotine. Simultaneously animals in the control subgroup of both groups received subcutaneous injection of 1 ml physiological saline. All animals were maintained in the same phase of estrous cycle before starting of the nicotine treatment.

**Sample collection:** After 15 days, the rats were kept fasting overnight and sacrificed by decapitation between 0900 - 1100 h. Blood was collected from heart
immediately after decapitation in heparinized tubes and plasma was separated by centrifugation and stored in vacuum desiccatator at -20 °C prior to further analysis. Ovaries were dissected out and wiped clear with tissue paper to remove adhering blood and tissue fluid, weighed and stored in vacuum desiccatator at -20 °C as such without pulverization or homogenization in buffer in order to prevent exposure to auto-oxidation environment.

Lipid profile test: The lipid components such as TC\textsuperscript{20} HDL-C\textsuperscript{21} and triglyceride\textsuperscript{22} were estimated in plasma by using standard kits supplied by Ranbaxy Diagnostic Limited, Mumbai, India. VLDL-C and LDL-C were calculated from the value of triglyceride, TC and HDL-C by Friedwald and Fredickson’s formula\textsuperscript{23}.

Lipid peroxidation assay: Plasma and ovary lipid peroxide were measured by the assay of thiobarbituric acid reactive substances (TBARS) according to the standard method\textsuperscript{24}. The amount of MDA was calculated by taking the extinction coefficient of MDA to be 1.56 x 10\textsuperscript{5} M\textsuperscript{-1} cm\textsuperscript{-1}.

Estimation of antioxidant enzymes: Antioxidant enzymes, superoxide dismutase (SOD)\textsuperscript{25} and catalase (CAT)\textsuperscript{26} of ovary were determined in rats under both normal and protein restricted dietary conditions.

Protein concentration of the tissue was determined by according to Lowry\textsuperscript{27}. The entire biochemical assays were repeated at least three times.

**Results**

The administration of nicotine in female rats caused a significant increase (\(P<0.01\)) of total cholesterol (11%)

<table>
<thead>
<tr>
<th>Table I. Effect of nicotine on plasma lipid profile of rat under different dietary conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Nicotine treated</td>
</tr>
<tr>
<td>Nicotine treated &amp; vitamin C supplemented</td>
</tr>
<tr>
<td>Nicotine treated &amp; vitamin E supplemented</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Nicotine treated</td>
</tr>
<tr>
<td>Nicotine treated &amp; vitamin C supplemented</td>
</tr>
<tr>
<td>Nicotine treated &amp; vitamin E supplemented</td>
</tr>
</tbody>
</table>

Values are mean ± SE from 10 animals in each group
* \(P<0.01\), ** < 0.001 compared to control
The data within the parenthesis represent the average percentage of increase (†) or decrease (‡) relative to the control.
Observed values of \(\chi^2\) lie in the acceptance region of it at 95 per cent level of significance.
with respect to control) in plasma in both dietary groups (Table I). Significant increase in plasma triglyceride, LDL-C ($P < 0.001$) in both diet groups and in VLDL-C in both dietary group were observed due to nicotine treatment. Vitamin C and E supplementation to the normal dieted rats reduced the nicotine-induced effects to some extent. In case of restricted dietary protein, the recovery effects of the two antioxidants, particularly vitamin E were poor (Table I).

Nicotine treatment caused a significant ($P < 0.001$) increase in plasma and ovary MDA levels of female rats under both dietary conditions (Table II), but the effect of nicotine was more pronounced in protein-restricted group. Ascorbic acid supplementation to the normal diet was more effective to reduce the nicotine-induced effect on MDA levels of both plasma and ovary. Supplementation of vitamin E showed less recovery effect as compared to vitamin C for reduction of nicotine-induced effect on MDA levels in all cases.

**Table II.** Effect of nicotine on malondialdehyde level in ovary tissue and in plasma of rats under different dietary condition

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal protein diet (18 % casein)</th>
<th>Protein restricted diet (5 % casein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA in plasma (n mol/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.45 ± 1.91</td>
<td>3.56 ± 1.12</td>
</tr>
<tr>
<td>Nicotine treated</td>
<td>4.81 ± 1.53**</td>
<td>5.60 ± 1.50**</td>
</tr>
<tr>
<td>(39.4% ↑)</td>
<td>(57.3% ↑)</td>
<td></td>
</tr>
<tr>
<td>Nicotine treated &amp; vitamin C supplemented</td>
<td>3.72 ± 1.72*</td>
<td>4.21 ± 2.20</td>
</tr>
<tr>
<td>(7.8% ↑)</td>
<td>(18.25% ↑)</td>
<td></td>
</tr>
<tr>
<td>Nicotine treated &amp; vitamin E supplemented</td>
<td>3.92 ± 2.21</td>
<td>4.44 ± 2.45</td>
</tr>
<tr>
<td>(13.6% ↑)</td>
<td>(24.72% ↑)</td>
<td></td>
</tr>
</tbody>
</table>

| MDA in ovary (n mole/mg protein) |                                  |                                     |
|----------------------------------|----------------------------------|                                     |
| Control                          | 16.5 ± 2.40                      | 16.9 ± 2.48                         |
| Nicotine treated                 | 21.1 ± 1.42**                    | 23.3 ± 2.52**                       |
| (27.9% ↑)                       | (37.9% ↑)                        |                                     |
| Nicotine treated & vitamin C supplemented | 18.1 ± 1.50                  | 18.8 ± 3.53                         |
| (9.7% ↑)                         | (11.2% ↑)                        |                                     |
| Nicotine treated & vitamin E supplemented | 18.3 ± 0.58                  | 19.5 ± 1.55                         |
| (10.9% ↑)                        | (15.4% ↑)                        |                                     |

Values are mean ± SE from 10 animals in each group
* $P < 0.01$, ** $< 0.001$ compared to control

The data within the parenthesis represents the average percentage of increase (↑) or decrease (↓) relative to the control.

Observed values of $\chi^2$ lie in the acceptance region of it at 95 per cent level of significance

Exposure to nicotine caused a more significant decrease in SOD and CAT activities in ovary tissue of rats under both diet groups. Supplementation of the two antioxidant vitamins to the protein restricted-diet was more effective to antagonize the nicotine-induced effect on SOD activities (Table III).

**Discussion**

Cigarette smoking has acute and long-term effects on various functions of female system but the nature of the effects varies with the dietary status. Venkatesan et al have shown that the levels of plasma TC, LDL-C, non-HDL-C and MDA significantly elevated in smokers in comparison with non-smokers. In an earlier study a significant increase in serum TC, phospholipids, and triglycerides as well as the amount of lipids associated with VLDL and LDL of nicotine-treated rats have been shown. The present findings of significantly increased levels of plasma TC, triglyceride, LDL-C and VLDL-C, and a significant decrease in plasma HDL-C...
of the nicotine treated rats under both dietary conditions were in agreement with the earlier studies. Cigarette smoking may induce functional and chemical change in the living systems. Low dietary intake of antioxidant vitamins, such as vitamin C and vitamin E, increases the risk of illnesses whereas high dietary intake seems to be protective. Vitamin E is known to be an effective antioxidant; it converts superoxide radicals, lipid peroxide radicals to less reactive form. Maritz & van Wyk have demonstrated that vitamin C protects against some of the effects of nicotine on lung development in a rat model. Our experimental results of plasma lipid profile due to nicotine treatment with vitamin C or vitamin E supplementation supported the previous observations, although the protective role of vitamin E against nicotine was comparatively less in case of restricted dietary protein. Also the adverse effect of nicotine on lipid profile increased with dietary inadequacy.

Enhanced level of tissue lipid peroxides in nicotine-treated rats has been shown to be accompanied by a significant decrease in the levels of ascorbic acid, vitamin E, reduced glutathione, glutathione peroxidase, superoxide dismutase and catalase. In the present study an increase in plasma and ovary in normal diet and protein restricted groups MDA levels reflected nicotine-induced oxidative stress. Also nicotine caused greater damage due to higher oxidative stress under protein-restricted diet, and the stress was partially removed when antioxidant vitamins were supplemented to the diet as previously described by Kim and Lee.

Significant reduction of antioxidant enzymes activities in ovary such as SOD and CAT suggested scavenging of free radicals from the ovary tissues by exposure of nicotine which was aggravated by metabolic stress under protein restriction condition. Increased production of free radicals or decreased function of the defense system play an important role in nicotine toxicity. Nicotine may thus potentiate the destructive oxidative stress in ovaries. Restoration of the activities of the antioxidant enzymes could be due to the capacity of the antioxidants to scavenge reactive oxygen species. The effectiveness of vitamin E as an antioxidants as shown earlier, is also supported by our result.

In conclusion, our findings showed that administration of nicotine caused significantly change in the plasma lipid profile, promoted lipid peroxidation in plasma and ovary tissue and significantly reduced ovarian antioxidant enzymes activities of female rats under protein-restricted condition. The supplementation of ascorbic acid or α-tocopherol antagonized the nicotine-induced effects though less effectively in protein-restricted condition.

Acknowledgment

Authors acknowledge the financial support received from R.D. Birla Smarak Kosh, Mumbai, India, and thank Dr D.K. Bhattacharji (Ex Emeritus Professor, Department of Chemical Technology, University of Calcutta) for comment, Shri T.K. Mandal for technical support.

References


*Reprint requests*: Dr Brajadulal Chattopadhyay, Department of Physics, Jadavpur University, Kolkata 700 032, India e-mail: bdc_physics@yahoo.co.in