Cigarette smoking is an important risk factor in the induction and the progression of cardiovascular diseases, lung cancer, and chronic obstructive pulmonary disease. Nicotine is a major toxic alkaloid compound present in smoke and smokeless tobacco (1). Nicotine promotes oxidative stress and oxidative damage in various organs and tissues from experimental models (2, 3). Short-term nicotine exposure induces myocardial contractile dysfunction and interstitial fibrosis through oxygen, reactive oxygen species (ROS) production and apoptosis (4)

Chronic nicotine treatment modulates the expression of cardiac genes involved in energy metabolism, signal transduction, and oxidative cardiac infraction (5). Oxidative stress seems to be an essential mechanism of nicotine toxicity. It occurs...
when the production of free radicals exceeds the scavenging effects of antioxidants, the depression of antioxidant status leading to peroxidation of lipids and the oxidation of proteins and DNA impairing both cardiac structure and function (2, 3). Nicotine activates the apoptosis that induces ischemic lesions (4, 6). Apoptosis has been shown to target mitochondria and endoplasmic reticulum. The ER is a membranous organelle involved in synthesis, folding, sorting and delivering proteins to their final cellular destination. The accumulation and aggregation of unfolded proteins induces ER stress, which initiates an adaptive mechanism named the unfolded protein response (UPR) to improve normal ER function and cell survival. If the ER stress is prolonged, the failure of UPR to restore the folding capacity leads to the activation of apoptosis (7). Nicotine induces ER stress in cultured lung cells (8, 9). The molecular mechanisms underlying the cardiotoxicity of nicotine have not been fully elucidated yet. Actually, whether ER stress is involved in the development of ischemic disease after nicotine exposure is not known. Epidemiologic studies suggest that Asian green tea consumption is associated with reduced mortality due to cardiovascular disease (10). Green tea is a potentially health-promoting beverage of dried leaves of *Camellia sinensis* because it contains relatively large amounts of polyphenols, mainly catechins with several biological functions, including anti-inflammatory (11), anti-oxidative (12) anti-carcinogenic and anti-proliferative properties (13). Therefore, the purpose of this study is to determine the effects of nicotine on cardiac structure, oxidative stress state, and indicating pathways in heart and whether GTE can attenuate nicotine cardiotoxicity. We have also investigated the expression of endogenous ER stress markers and MAP kinase in cardiac cells exposed to nicotine in the presence or the absence of epigallocatechin gallate (EGCG), the major component of polyphenols in green tea.

**MATERIALS AND METHODS**

**Experimental animals**

The researchers obeyed the “guide for the care and use of laboratory animals”. Thirty-two adult male albino rats were housed in a temperature-controlled room, receiving water and food *ad libitum*. They were acclimatized under laboratory condition for one week prior to experiments. The rats were randomly divided into four groups of 8 rats. Group I served as control (C). Group II, control tea (CT) served as control and received GTE (2%, w/v) orally, as the only drinking fluid. Group III, nicotine treated rats (N) were injected with subcutaneously single dose of nicotine (1 mg/kg b.w.). Group IV, nicotine tea treated rats (NT) received subcutaneously single dose of nicotine (1 mg/kg b.w.) and GTE (2% w/v) concomitantly. During the experimental period, the body weight was monitored every week. Then, after 2 months of treatment, the rats were fasted overnight. Blood samples were collected from each rat through the puncture of the retro-orbital sinus into sterile tubes without anticoagulant, centrifuged at 1500 ◦ g for 15 min at 4°C to obtain serum, and stored as aliquots at -20°C. The animals were scarified under light ether anesthesia. The hearts were removed from the rats and immediately rinsed in PBS. The ratio of heart weight to body weight (cardiac mass index) was measured for each rat. The hearts were cut into small pieces on ice, rapidly frozen in liquid nitrogen, and stored at -80°C for later analysis.

**Green tea extract**

Twenty grams of green tea dry leaves were soaked in 1 L of boiling water for 10 min and filtered to make a 2% instant green tea solution. The filtrate designated as green tea extract (GTE) was provided to rats as their sole source of drinking water. Green tea (*C. sinensis*) was imported from China.

**Preparation of nicotine**

Hydrogen tartrate salt of nicotine (Sigma, N5260-25G) was dissolved in 0.9% NaCl solution. The pH of nicotine solution was adjusted to 7.4 by 0.2 M NaOH.

**Biochemical parameters and cardiac markers**

The levels of specific markers related to cardiac dysfunction CPK (creatine phosphokinase), CPKMB (creatine phosphokinase muscle brain), lactate dehydrogenase (LDH), total cholesterol, LDL cholesterol levels and liver tissue injury markers (alanine aminotrasferases - ALAT and aspartate aminotrasferases - ASPAT) were estimated using standard commercial kits and an automatic biochemistry analyzer.

**Histological study**

A portion of heart tissues from control and experimental animals were fixed in 10% formalin at room temperature for 24 h. The specimens were dehydrated in gradient series of ethanol, cleared in xylene, and embedded in paraffin. Sections about 3 µm thick were stained with hematoxylin-eosin or Masson trichrome to be evaluated with light microscope.
**Estimation of malondialdehyde (MDA)**

The assay for lipid peroxidation in serum and heart homogenates was done according to method (14). The MDA levels were measured in serum and tissue homogenates by evaluating the reaction between MDA and thiobarbituric acid (TBA). About 2 mg of rat heart were cut into small pieces into lyse buffer, and the homogenates were centrifugated at 10000 × g for 20 min at 4°C. Two hundred forty µL of the supernatants were mixed with 600 µL of trichloroacetic acid (TCA) 20% in order to discard proteins and 500 µL of TBA was added to supernatant. For serum level evaluation of MDA, 200 µL of serum were used. The mixture was heated at 90°C for 15 min, cooled back on ice for 5 min, and centrifuged at 10000 × g for 10 min at 4°C. The absorbance was read at 530 nm. The amount of MDA was expressed as µmole of MDA formed per minute.

**Enzyme and proteins assays**

Catalase activity was measured according to (15). The reaction mixture (2 mL) contained 0.1 M phosphate buffer (pH = 7), 0.091 M H$_2$O$_2$ and heart homogenate. The reaction started by adding H$_2$O$_2$ and its decomposition was monitored at 560 nm. The absorbance was recorded after 1 min during 2 min. The enzyme activity was calculated in the international enzymatic unit (UI). The protein content was estimated according to (16) using the bovine serum albumin as standard. Catalase activity was expressed on UI /mg /min.

**Isolation of rats cardiomyocytes and in vitro drug treatment**

Adult rats (250-280 g) were anesthetized using light ether. The hearts were rapidly removed and maintained in DMEM (Dulbecco’s modified Eagles medium) supplemented with 10% FCS (fetal bovine serum) and 5% HEPES [4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid]. The ventricles were removed and minced in DMEM supplemented with 10% FCS, 10% glutamine 200 mM, and 1% antibiotic (streptomycin at 50 µg/mL; penicillin at 50 µg/mL) at 37°C in air with CO$_2$ (95/5%) atmosphere, until confluence. The cardiomyocytes were then trypsinized and subcultured. For the experiments, the cardiomyocytes were cultured in 12-well plates. The cells were randomly divided into 4 groups: cells cultured in normal culture media (the control group); cells treated with EGCG at 50 µM (EGCG group); cells incubated with nicotine (10 µM) alone at 37°C for 24 h (nicotine group), or in the presence of EGCG at 50 µM (nicotine + EGCG group). In the present experiment, nicotine concentration was fixed at 10 µM because (6-17) have reported this amount as the nicotine plasma level in smokers. Zhou et al. (6) have reported that EGCG at 50 µM as a cardioprotective and healthy dose. EGCG was purchased from Sigma Aldrich Chemie (E414350MG).

**Western blot analysis**

Total protein samples from myocardium tissues and cells were homogenized in a Bertin homogenizer with a buffer containing orthovandate (1 mM), DTT (dithiothreitol) (10 mM), NaF (50 mM), NaPPi (sodium pyrophosphate inorganic tetrasac) (5 mM), PMSF (phenylmethanesulfonyl fluoride) (2 mM) and protease inhibitor cocktail (Calbiochem). The protein concentration of supernatant was determined using BCA protein assay. Fifty µg of lysate were separated by electrophoresis on 12% SDS-polyacrylamide gels under reducing conditions and transferred onto nitrocellulose membrane. After blocking the membranes with blocking buffer containing 5% non fat milk in TBS-T (Tris buffered saline-Tween) for 1 h, the membranes were incubated with the following antibodies: anti-phospho ERK 1/2 (1 : 1000), anti-GRP78 (1 : 1000), anti-AIF (1 : 1000), anti-HSP70 (1 : 500), anti-CHOP (1 : 100), anti-p-p38 (1 : 500), VDAC (1 : 1000). Membranes were washed three folds in TBST for 15 min at room temperature and then incubated with horseradish peroxidase-conjugated secondary antibody, either anti-mouse (1 : 1000), anti-rabbit (1 : 1000) and anti-goat (1 : 1000) for 1 h at room temperature. The antibodies were purchased from Santa Cruz Biotechnology, Cell Signaling Technology and Abcam. The bands were visualized with chemidoc (Bio-Rad Laboratories, Canada) and quantified by image analysis software (quantity one, Bio-Rad Laboratories, Canada). The optical density values were expressed as arbitrary units (AU). The anti β-actin antibody was used to show equal loading of the protein.

**Statistical analysis**

All data are expressed as the mean ± S.D. Data thus obtained from each group were tested by one-way ANOVA with post-hoc test for multiple comparisons. The p ≤ 0.05 value was considered statistically significant. SPSS17.0 for Windows was used for statistical analyses.

**RESULTS**

No statistical difference in body weight and cardiac mass index were observed among treated
and control groups. The parameters were similar among all four groups (Table 1).

Significant increase of total cholesterol (p < 0.01), LDL-cholesterol (LDLc) (p < 0.01) and triglycerides (p < 0.001), were observed in nicotine-submitted group compared to control and control tea (GTE). Supplementation attenuated but not significantly (p > 0.05) nicotine induced toxic effects as observed in Table 2.

The changes in the activities of the plasma dehydrogenase lactate (LDH) and CPK and CPKMB are given in Table 3. The activities of this

### Table 1. Growth parameters in all experimental groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Control tea</th>
<th>Nicotine</th>
<th>Nicotine tea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>239.25 ± 30.34</td>
<td>296.66 ± 42.83</td>
<td>290.66 ± 42.79</td>
<td>315 ± 53.50</td>
</tr>
<tr>
<td>Heart weight index</td>
<td>3.21 × 10-3 ± 0.00065</td>
<td>3.09 × 10-3 ± 0.00037</td>
<td>2.92 × 10-3 ± 0.00028</td>
<td>3.06 × 10-3 ± 0.00080</td>
</tr>
<tr>
<td>Liver weight index</td>
<td>0.028 ± 0.0010</td>
<td>0.026 ± 0.0018</td>
<td>0.024 ± 0.0003</td>
<td>0.027 ± 0.0031</td>
</tr>
</tbody>
</table>

Data represent the mean ± S.D.

### Table 2. Plasma lipid profile and cardiac markers in different groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Control tea</th>
<th>Nicotine</th>
<th>Nicotine tea</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-Cholesterol (mmol/L)</td>
<td>0.26 ± 0.04</td>
<td>0.24 ± 0.07</td>
<td>0.57 ± 0.23**a,**b</td>
<td>0.37 ± 0.19**</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>0.07 ± 0.015</td>
<td>0.08 ± 0.025</td>
<td>0.23 ± 0.110**a,**b</td>
<td>0.12 ± 0.007**</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>0.18 ± 0.060ab</td>
<td>0.18 ± 0.059</td>
<td>0.58 ± 0.064**a,**b</td>
<td>0.419 ± 0.060**a,#b</td>
</tr>
<tr>
<td>LDH (UI/L)</td>
<td>19.6 ± 5.37</td>
<td>40.80 ± 4.76</td>
<td>442.25 ± 60.36**a,#b</td>
<td>218,2 ± 39.50**a,#b,#c</td>
</tr>
<tr>
<td>CPK (UI/L)</td>
<td>138.4 ± 34.6</td>
<td>53 ± 4.5</td>
<td>1729 ± 153.8**a,#b</td>
<td>156,5 ± 44.7#b,#c</td>
</tr>
<tr>
<td>CPKMB (UI/L)</td>
<td>175.2 ± 48.5</td>
<td>177.5 ± 13.6</td>
<td>480.8 ± 64.8**a,#b</td>
<td>222,19 ± 53.02**a,#b,#c</td>
</tr>
</tbody>
</table>

Values are the mean ± S.D. (a), (b), (c) corresponded respectively to the groups control, control tea and nicotine. * a p < 0.05 compared to nicotine; **b, **c p < 0.01 comparing to control and control tea; ***a, ***b p < 0.0001 compared to control, control tea and nicotine.

### Table 3. Malondialdehyde levels in different groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Control tea</th>
<th>Nicotine</th>
<th>Nicotine tea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac MDA (µmol/mL)</td>
<td>0.088 ± 0.01</td>
<td>0.040 ± 0.02**a</td>
<td>-0.120 ± 0.02**a,#b</td>
<td>0.019 ± 0.07**a,#b,#c</td>
</tr>
<tr>
<td>Plasma MDA (µmol/ mL)</td>
<td>0.026 ± 0.007</td>
<td>0.046 ± 0.003</td>
<td>-0.400 ± 0.1**a,#b</td>
<td>0.126 ± 0.058**a,#b,#c</td>
</tr>
</tbody>
</table>

Values are the mean ± S.D. (a), (b), (c) correspond respectively to the groups control, control tea and nicotine. * a p < 0.05 compared to control; **a p < 0.01 compared to control tea and nicotine; ***a p < 0.0001 compared to control, control tea and nicotine.

### Table 4. Enzymatic activity in different groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Control tea</th>
<th>Nicotine</th>
<th>Nicotine tea</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (UI/L)</td>
<td>16.98 ± 2.44</td>
<td>10.39 ± 3.11**a</td>
<td>46.60 ± 3.2**a,#b</td>
<td>26.85 ± 2.64**a,#b</td>
</tr>
<tr>
<td>ASLT (UI/L)</td>
<td>16.96 ± 4.5</td>
<td>4.50 ± 0.7**a,#b</td>
<td>32.58 ± 2.5**a,#b</td>
<td>16 ± 3.15**a,#b</td>
</tr>
<tr>
<td>Catalase (UI/ min /g)</td>
<td>0.43 ± 0.067</td>
<td>2.79 ± 0.975**a,#b</td>
<td>-0.17 ± 0.04**a,#b</td>
<td>2.65 ± 1.01**a,#b</td>
</tr>
</tbody>
</table>

Values are the mean ± SD. (a), (b), (c) correspond respectively to the groups control, control tea and nicotine. * a p < 0.05 compared to control, control tea and nicotine.
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Figure 1. Western blot analysis of P38, ERK 1/2 MAPK and chaperons HSP70 and GRP78 in heart rat in different experimental conditions: control (C), GTE-treated control (CT), nicotine-submitted group (N), and group submitted to nicotine and treated with GTE (NT) for two months. MAPK and chaperons were characterized by immunobloting and represented in (Fig. 1A). Figs. 1B and 1C correspond to the quantitative data. Each bar indicated the mean ± S.E.M of three different blots. Signals were quantified by densitometric analysis of the relative protein levels and are expressed as a percentage of the control in heart in different groups: # one-way ANOVA post hoc test p < 0.0001, *one-way ANOVA post hoc test p ≤ 0.05. Comparing to control group, the phosphorylation of p-P38 increased (p < 0.0001) in all experimental groups. After administration of GTE, the amount of p-ERK 1/2 increased significantly (p < 0.0001) in nicotine-treated group compared to control and group treated only with nicotine. The expression of GRP78 and HSP70 were significantly expressed after the administration of nicotine alone or with GTE.
enzymes were significantly increased ($p < 0.0001$) in nicotine submitted group when compared with control and control tea. The co-treatment with GTE induced more significant decrease ($p < 0.0001$) of LDH, CPK and CPKMB activities.

The changes in lipid peroxidative product levels, MDA are summarized in Table 3. The levels of cardiac MDA were more significantly increased ($p < 0.0001$) in nicotine treated rats when compared with control tea. The plasma MDA level of nicotine treated groups were highly and significantly increased ($p < 0.0001$) compared to control and control tea. The GTE co-treated group showed significant decrease in levels of both heart and plasma MDA ($p < 0.01$, $p < 0.0001$).

Nicotine treatment affected biochemical markers of liver function, as shown in Table 4. Seric ALT and AST levels showed significant increase ($p < 0.0001$) in nicotine treated rats when compared with control and control tea. A significant decrease of plasma levels of ALAT and ASPAT levels ($p < 0.0001$) was observed in control tea compared to control. The GTE co-treatment in nicotine-treated rats induced significant decrease ($p < 0.0001$) in ALAT and ASPAT levels compared to nicotine treated rats. The activity of the catalase, antioxidant enzyme, is highly significantly decreased in nicotine treated group when compared to control tea group ($p < 0.0001$). A significant increase of catalase activity in the control tea group was observed compared to the control group. The administration of GTE to the nicotine treated group increased significantly ($p < 0.0001$) the catalase activity compared to nicotine group.

![Western blot analysis of cardiac apoptotic proteins, AIF and CHOP in rat of all groups (C, CT, N, NT). The immunoblotting was indicated in Figure 2A and the quantitative data in Figure 2B. Each bar represented the mean ± S.E.M. of three blots. Signals were quantified by densitometric analysis and are expressed as a percentage of the control. The amount of CHOP and AIF increased in nicotine and nicotine tea groups ($p < 0.0001$) compared to control and control tea.](image)
Results indicated in Figure 1 A and B have revealed the activation of MAPK, P38 and ERK 1/2 in myocardium following nicotine and GTE administration. The proteins levels expression of P38 and ERK 1/2 in control tea, nicotine and nicotine tea groups were compared to controls. The ERK 1/2 is upregulated in control tea and nicotine-tea groups, a significant increase (p < 0.0001) of this protein was observed in nicotine-tea group and control tea compared to control. The p-p38 is highly expressed (p < 0.0001) in control tea, nicotine and nicotine tea groups compared to control.

The activation of ERS is one of the important mechanisms that induce cells apoptosis during cardiac lesions. As indicated in Figure 1A and 1C, our results revealed significantly up regulated expression of ER stress markers GRP78, HSP70 in nicotine and nicotine tea rats hearts (p< 0.0001) compared to control and control tea. When GTE was given to nicotine-treated rats, it increased the expression levels of this chaperons (p < 0.0001, p < 0.001). As shown in Figures 2 A and B, the expression of apoptotic proteins CHOP and AIF was markedly up regulated in nicotine-treated rats (p < 0.0001) compared to control and control tea. The co-treatment of the nicotine treated rats with GTE, attenuated significantly (p < 0.0001) the expression of the two proteins compared to the rats group treated with nicotine alone. As GTE attenuated the expression of AIF, ER stress proteins and enhanced MAPKs expression, we examined the expression of ER stress markers and MAPKs in cardiomyocytes submitted to nicotine and EGCG. As shown in Figure 3, the expression of ER stress proteins, GRP78, HSP70 and CHOP were markedly expressed in cardiomyocytes exposed to nicotine. The phosphorylation of p38 increased in all groups compared to the control group. However, the phosphorylation of ERK 1/2 increased only in EGCG group and EGCG + nicotine group.

Histological examination of heart sections from rats exposed to nicotine revealed marked tissue damage and changes (Fig. 4). The changes included interstitial edema, irregular cardiacmyocytes shape, angiogenesis, lymphocytes infiltration and myocardial fibrosis. GTE treatment markedly reduced the degree of injuries.

DISCUSSION

Smoking and nicotine are the major risk factors for the development of cardiovascular disease that may lead to alter the normal plasma lipoproteins pattern. In the present finding, we have showed significant increased levels of TC, TG and a significant increase of LDL C of nicotine submitted group. Previous study has showed the same lipid profile after nicotine treatment of the female rats (18). Our
results have showed no significant changes in body weight and heart mass index in all experimental groups. Previous studies have reported that nicotine administration has decreased body weight and food intake (19). We can suggest that the effects of nicotine on the body weight, heart mass index and biochemical parameters are time and dose dependent. A strong relationship between oxidative stress and cardiotoxicity has been speculated in many experimental models (20). Nicotine has been reported to induce oxidative stress by increasing the levels of MDA, a stable product of free radicals species that attack lipids component, leading to lipids peroxidation and decreasing the activity of scavenging enzyme such as catalase and superoxide dismutase (21, 22). In our study, nicotine administration was associated with an oxidative stress increase as supported by an increase in levels of myocardial and serum MDA and a decrease of the endogenous antioxidant enzyme catalase activity. The GTE co-treatment has reduced the levels of MDA and increased the catalase activity compared to the controls. This result has indicated that GTE can prevent the nicotine toxicity by inhibiting the peroxidation of lipids and improving the activity of the antioxidant enzyme catalase. Oxidative stress induces cellular damage and leakage of cytoplasmic markers in circulation such as CPK, CPKMB, LDH and the transaminases. It has been reported that the serum levels of this enzymes is elevated during cardiac lesions (22, 23). The plasmatic levels of the cardiac markers were significantly reduced with GTE co-

Figure 4. Photomicrographs of rat myocardium (My) stained with H&E or Masson’s trichrome showed the effects of GTE supplementation after nicotine treatment. (A) (40x) and (B) (40x) Rat heart sections from control and control tea (2% w/v, oral for 2 months) revealed the presence in the myocardium (My) of cardiomyocytes (C) with regular nuclei (Cn). (C) (40x) and (D) (100x) hearts sections from nicotine-treated animals (1 mg/kg, i.p. for 2 months) showed the presence of disorganized and irregular chap cardiomyocytes (Cd), fibroblastes nuclei (Fn), interstitial edema (Id), angiogenesis (A) and accumulation of conjunctive material (CM) around the vessels (V) accompanied by inflammatory cell (ICs) infiltration. (E) In heart sections (40x) from rats treated with nicotine (1 mg/kg, i.p. for 2 months) and GTE (2% w/v oral for 2 months) a significant structural amelioration is observed.
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treatment in nicotine group. Elevated transaminases levels reflect the liver damage and deleterious effects of nicotine. The present study provided evidence for the first time that green tea attenuates nicotine cardiotoxicity. High performance liquid chromatography analysis of 2% green tea infusion given as the sole source of drinking water to rats revealed the presence of a high levels of polyphenols, EGCG and ECG (13). In vitro electron paramagnetic resonance study of EGCG or GCG reveals directly quench activity of active oxygen radicals which demonstrates the strongest antioxidant activity of 2% GTE rich in polyphenols (12). In this report, we showed that nicotine induces cardiac ischemic lesions revealed by structural alterations, fibrosis, inflammatory cell infiltration, angiogenesis and degeneration of cardiomyocytes were seen. We have observed that oral administration of GTE attenuates the myocardial lesions. Structural observation has confirmed the cardioprotection nature of green tea extract. Catechins attenuate myocardial injury by several mechanisms, such as the suppression of proinflammatory factors and adhesions molecules (11) inhibition of STAT1 (Signal transducer and activator of transcription 1) activity (24) and antioxidant activity (12). The ischemic lesions target the mitochondria and alter the mitochondrial electron transport, which activates the caspase dependent and independent cell death (26), have shown that the reversible blockade of electron transport before ischemia protects mitochondria against cardiac damage by preventing the release of apoptosis inducing factor (AIF) from mitochondria, which leads to decreased activation of PARP-1, the later known to activate caspase independent cell death by inducing DNA fragmentation and chromatine condensation. In this study, we have found that the levels of AIF expression was increased clearly when rats were treated with nicotine. The levels of AIF were significantly decreased by co-treatment with GTE. These results can suggest that nicotine-induced apoptosis via mitochondrial release of AIF known to be apoptogenic.

Several works have shown that nicotine and green tea extract activate mitogen-activated protein kinases in vivo and in vitro (26, 27) and the MAPK was implicated in the cellular defensive mechanisms during stress. In this study, we have analyzed by immunoblot the effects of nicotine and GTE on MAPK (ERK 1/2, P38) signaling pathways and our results have indicated that nicotine action has significantly increased the phosphorylation of P38 whereas the phosphorylation of ERK 1/2 was decreased. The P38 mitogen-activated protein kinase also is known as stress-activated protein kinase implicated in proliferation, differentiation and cells death regulation in heart. P38 presents a protective and detrimental activity during myocardial ischemia. These effects should be dependent on the system studied and the activated P38 isoforms (28-30). Whereas the activation of the signal-regulated protein kinase ERK 1/2 has been linked with hypertrophic and anti-apoptotic response in heart and in cultured cardiomyocytes (20-31). Our results have revealed the role of MAPK stress signaling cascades in nicotine-induced cardiac dysfunction. The expression of p38 MAPK after nicotine and GTE treatment reflects heterogeneity in the expression and/or activation of p38 isoforms. The marked increase of ERK 1/2 phosphorylation after GTE administration suggests that this redox-sensitive kinase is involved in the hypertrophic response that reduced the heart injury. Cigarette smoking and nicotine induce ERS (26-32). Our result revealed a significant expression level of chaperons stress markers GRP78, HSP70 after nicotine treatment. GRP78 is the ER resident chaperon reported in ER stress-induced in mouse heart after transverse aortic constriction (33), in vivo myocardial infarction (34) and neonatal cardiomyocytes submitted to hypoxia (35). HSP70 also plays a cytoplasmic role in rat heart. In a rat model of myocardial ischemia, reperfusion injury, neonatal cardiomyocytes submitted to stress condition (36), induction of HSP70 correlates with reduction of cell death. The induction of HSP70 and GRP78 after nicotine administration may be reflect their roles during ER stress in modulating polypeptides folding, degradation and translocation across membranes and protein–protein interactions. The co-treatment with GTE reduces the ER stress and the expression of the both chaperons may preserve the cells survive. However, (37) has reported that tea polyphenol, EGCG induces ER stress in the hepatoma cells Hepa1c1c7 by the activation of programmed cell death and ER-derived proapoptotic signals, CHOP-induction and procaspase-12 cleavage. Several studies have shown that cigarette smoke and nicotine also trigger apoptosis through oxidative stress and ER stress induction of CHOP (8-26). To establish the link between the ERS and apoptosis, we examined the levels of proapoptotic transcriptional factor C/EBP homologue protein, CHOP also known as growth arrest and DNA damage inducible protein 153 (GADD153). Our results demonstrate that the administration of GTE attenuated the heart expression of CHOP in nicotine treated rats. CHOP is shown to mediate apoptosis by down-regulating of Bcl2 (38), perturbing the cellular redox state and facilitates the translocation of
proapoptotic protein Bax (39). The supplementation with green tea protects against ERS-initiated apoptosis in heart of nicotine treated rats. Green tea and its polyphenol EGCG possess antiapoptotic activity in several experimental models, which is attributed to its ability of blocking signals transduction pathways leading to cell death (11, 12-24). In conclusion, the present study provided evidence for the first time that oral administration of green tea attenuates nicotine cardiotoxicity. This cardioprotective effects may be achieved by increasing the activities of antioxidative enzyme and attenuating malondialdehyde contents and ER stress induced apoptosis. The nutritional antioxidants supplementation attenuates the tissues damage caused by nicotine. Clinical studies were warranted to investigate the effects of green tea beverage on subject submitted to nicotine and cigarette smoking and these findings bring an insight to the smokers.

Conflict of interest statement

We declare that we have no conflict of interest.

REFERENCES


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