Alpha-Lipoic Acid Improves Antioxidant Capacity but Has No Blood Pressure Lowering Effect in Rats Administered Nicotine

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ABSTRACT
Background: Nicotine has been implicated as a major player in smoking-induced cardiac toxicity. Alpha-lipoic acid (ALA) is a unique and potent antioxidant that scavenges reactive oxygen species with the ability to regenerate other antioxidants.
Objective: Oxidative stress is thought to play a fundamental role in nicotine toxicity; this study therefore, investigated the effect of dietary supplementation with ALA on nicotine-induced cardiac toxicity.
Methods: Twenty-eight (28) male Sprague-Dawley rats (150-200 g) were divided into 4 groups of 7 rats each of control, nicotine only, nicotine + ALA, and ALA only. Nicotine (0.5 mg/kg i.p.) and ALA (200 mg/kg) were administered for four weeks and the animals were fed with rat chow ad libitum. Rats were then anaesthetized with urethane and α-chloralose (5 ml/kg/b.w. i.p.); blood pressure recordings were obtained by the cannulation of left carotid artery connected to a pressure transducer and a Power-lab system. Blood samples were withdrawn for biochemical analysis.
Results: The study showed increased systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressures (MAP), and rate pressure product (RPP) in nicotine administered rats and nicotine + ALA group compared to control. Increased levels of C-reactive protein (CRP), malondialdehyde (MDA) and reduced levels of antioxidant enzymes (glutathione peroxidase [GPx] and superoxide dismutase [SOD]) were observed in the nicotine group. No significant change was found in the lipid profile across the groups. Supplementation with ALA (Group 3) reduced the elevated MDA levels and increased SOD and GPx levels in the nicotine treated rats but did not affect blood pressure.
Conclusion: This study showed that ALA suppressed oxidative stress but not blood pressure in rats administered nicotine.
Keywords: Nicotine, alpha-lipoic acid, blood pressure, antioxidant, oxidative stress.

INTRODUCTION
The cardiovascular system is very crucial to the optimal functioning of the body and the total quality of life. It supplies oxygen from the lungs to the tissues around the body and transports carbon dioxide, a waste product, from the body to the lungs amongst other functions. Harmful lifestyle habits such as smoking, being inactive, overweight, poor dieting and excessive alcohol intake set the stage and spurs on cardiovascular diseases such as stroke, peripheral artery disease, valve problems, aortic aneurism and heart failure (1).

The damage by these harmful habits could also extend to other parts of the body such as the kidneys, bones and the brain (1).

Smoking either actively or passively adversely affects the cardiovascular system in human subjects. Although approximately 4000 components exist in the cigarette, nicotine is the alkaloid most active in tobacco (2–3). Higher concentrations of nicotine in tobacco and cigarette smoke have been implicated as a major player involved in smoking induced toxicity (4). One cigarette delivers about 1.2–2.9 mg of nicotine, amounting to about 20–40 mg of nicotine per day in a typical one pack per day smoker (5). Each puff contains approximately 50 mg of nicotine (6). Nicotine exerts its cardiovascular effect via sympathetic neural stimulation resulting in increased heart rate, blood pressure, increased blood flow and cardiac output, and increased myocardial oxygen demand (6–8).

Nicotine is associated with increased risk of cardiovascular diseases such as atherosclerotic vascular disease, hypertension, myocardial infarction, unstable angina, sudden cardiac death, and stroke (9). This could be a result of a series of interdependent processes, such as enhanced oxidative stress, inflammation, hyperlipidaemia, haemodynamic and autonomic alterations, endothelial dysfunction, and thrombosis (10). Nicotine triggers an increase in levels of free radicals or reactive oxygen species (ROS) which causes oxidative stress, damages cell membranes and causes tissue damage (11–12). The ROS produced in turn, activates redox-sensitive nuclear transcription factor kappaB (NF-kappaB), which is involved in various biological processes including inflammation and cell death (13–14).

Alpha-lipoic acid is a unique and potent antioxidant. It
can deliver antioxidant activity in both fat and water-soluble mediums (15). This effectively allows ALA to deliver its antioxidant effect to any cell or tissue type, as well as to any subcellular compartment in the body (16–17). Oxidative stress is known to activate redox-sensitive cellular signalling pathways, which in turn has been implicated in several cardiovascular dysfunctions such as hypertension, atherosclerosis, and heart failure (18–19). The antioxidant properties of ALA are based on its ability to directly scavenge ROS, its metal chelating activity, and its potential to react with and regenerate other antioxidants such as glutathione and vitamins E and C (20–21). Many studies on animal models have confirmed ALA can prevent progressive remodelling and even improve cardiac function (22–23).

Alpha-Lipoic acid may have a beneficial effect in preventing the development of hypertension by lowering the level of inflammatory cytokines in the blood plasma, thus preventing pathological changes to vessel cells and normalizing changes in blood pressure (24). In one study (25), ALA significantly reduced levels of C-Reactive Protein (CRP), a risk factor for cardiovascular disease, in patients with renal disease. Some studies have shown a link between oxidative stress in the brain and increased sympathetic activity and hypertension (26–28). Since nicotine acts by activating the sympathetic nervous system, and oxidative stress plays a fundamental role in nicotine toxicity, there is a probability that antioxidants might help reduce sympathetic activity and thus lower blood pressure. This present study therefore explored the influence of ALA on nicotine-induced cardiac toxicity.

MATERIALS AND METHODS

Animals

Twenty-eight (28) male Sprague-Dawley rats weighing 150–200 g were obtained from the Laboratory Animal House of the College of Medicine of the University of Lagos. The rats were placed in well-ventilated plastic cages, which had been bedded with wood shavings and they were left to acclimatize for two (2) weeks in the departmental animal room before the study commenced. They were fed with standard pellet diet and water ad libitum under standard conditions. After two weeks of acclimatization, the rats were then randomly divided into 4 groups of 7 rats each and administered ALA and nicotine for 4 weeks. All experimental procedures were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Groupings

Seven rats were randomly assigned into four groups of: Group 1 (control), Group 2 (nicotine treatment), Group 3 (nicotine + ALA treatment), and Group 4 (ALA treatment). Rats from Groups 2 and 3 were given nicotine intraperitoneally (i.p.) at a dose of 0.5 mg/kg body weight as a solution (29). ALA was dissolved in distilled water to form a solution of 20 mg/ml and administered at a dose of 200 mg/kg body weight (30) orally via an oral cannula to rats in Groups 3 and 4. All treatment lasted for four consecutive weeks.

Sample Collection and Blood Pressure Measurement

The rats were anaesthetized with a mixture of 25% (w/v) urethane and 1% (w/v) á-chloralose injected intraperitoneally at a dose of 5 ml/kg body weight. After administration of the anaesthetic agent, the animal that had lost its righting reflex was picked and placed supine on the dissecting board, limbs were fastened to the board, and the trachea was exposed and cannulated. The carotid artery was then cannulated and the cannula was connected to the pressure transducer (model SP 844, Physiological pressure transducer; AD Instruments) that was connected through MLAC11 Grass adapter cable to a computerized data acquisition system with LabChart-7 pro software (Power Lab-4/24 T, model MLT844/P; AD Instruments Pty Ltd., Castle Hill, Australia). The LabChart-7 pro software computes the heart rate (HR) by applying the cyclic measurement function, which is a channel calculation that analyses periodic blood pressure waveforms in real-time. Data of the detected cycles are displayed as continuous data-trace for HR in another channel of the data acquisition system. Recordings were taken at a sampling frequency of 5 Hertz. Then, the transducer was removed from the cannula and blood was collected into lithium heparinized and plain sample bottles.

Oxidative Stress Assessment

Lipid Peroxidation

Malondialdehyde (MDA) was determined as described by Sharma (31). 1.0 ml of the supernatant was added to 2 ml of TCA-TBA-HCl (1:1:1 ratio) reagent (thiobarbituric acid 0.37%, tricarboxylic 15%, 0.24N HCl) boiled at 100°C for 15 min. and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance read at 535 nm against a blank. MDA was calculated using the molar extinction coefficient for MDA-TBA complex of 1.56 × 105 M⁻¹ CM⁻¹.

Activity of Antioxidant Enzymes

Superoxide Dismutase (SOD) Activity

Superoxide dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480 nm (32). The reaction was carried out in 0.05 m sodium carbonate buffer pH 10.2 and was initiated by the addition of 3 × 10⁻⁴ epinephrine in 0.005 N HCl. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min. SOD activity was then expressed as (U/mg protein).

Catalase Activity

Catalase activity was determined according to the method of Beers and Sizer as described by Usoh (33) by measuring the decrease in absorbance at 240 nm due to the decomposition of H₂O₂ in a UV recording spectrophotometer. The reaction mixture (3 ml) contained 0.1 ml of serum in phosphate buffer (50 mM, pH 7.0) and 2.9 ml of 30 Mm H₂O₂ in phosphate buffer pH 7.0. An extinction coefficient for H₂O₂ at 240 Nm of 40.0 M⁻¹ cm⁻¹ was used for the calculation. The specific activity of catalase was expressed as moles of H₂O₂ reduced per minute per mg protein.
Glutathione Peroxidase (GPx) Activity
The activity of glutathione peroxidase (GPx) was measured as described by Pari (34). The glutathione peroxidase determination method is based on the reaction of Ellman's reagent. 1.0 ml of supernatant was treated with 0.5 ml of Ellman's reagent (19.8 mg of 5,5'-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2M, pH 8.0). The absorbance was then read at 412 nm spectrophotometrically and GPx activity was expressed as U/mg protein.

Lipid Profile Analysis
The lipid profile assay for total cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were carried out on a fully automated analyser based on spectrophotometric principle using kits obtained from ERBA diagnostics (Transasia Bio-Medicals Ltd., Germany) as described (35). The system employs wet chemistry and photometric technology to perform absorbance readings from the colour of the sample. The absorbance is then converted automatically into concentrations based on the standard calibration curves stored by the instrument’s microprocessor.

Determination of C-Reactive Protein (CRP) Levels
The biochemical analysis of CRP was done using high sensitive CRP turbilatex agglutination kit manufactured by Agappe (Switzerland) and supplied by NUMS Diagnostic Nigeria Ltd.

Statistical Analysis
All the values are expressed as mean ± standard error of mean (SEM). The values were analysed by one-way ANOVA followed by Student's Newman-Keuls post-hoc test using the Graph Pad Prism 6 software. Differences were considered significant when p<0.05.

RESULTS
Blood Pressure, Heart Rate and Rate Pressure Product in Rats
Systolic, diastolic and mean arterial pressures, as well as rate pressure products, were significantly increased (p<0.05) in both the nicotine group and in the nicotine + ALA group when compared to control rats. However, there was no significant difference (p<0.05) when compared to each other. Also, a significant decrease in the SBP was observed in the ALA treated group when compared to the nicotine and nicotine + ALA groups. No significant difference was observed in the HR and pulse pressure in all the groups.

Lipid Peroxidation
Figure 1 shows the effect of ALA on malondialdehyde (MDA) level (µmol/ml) in nicotine treated rats. Malondialdehyde level was significantly increased (p<0.05) when the nicotine group (0.028 ± 0.003 µmol/ml) was compared to control (0.016 ± 0.007 µmol/ml). However, co-treatment of ALA with nicotine resulted in a total reduction of these elevations (0.013±0.003 µmol/ml) to levels seen in the control group.

Antioxidant Enzymes

SOD Activity
Superoxide dismutase concentration was significantly reduced when the nicotine group (1.17±0.02 µmol/ml/mg protein) was compared to control groups (1.53±0.09 µmol/ml/mg protein). The observed increase in SOD levels in the nicotine + ALA group (1.38±0.11 µmol/ml/mg protein) was not significant when compared with nicotine group only (1.17±0.02 µmol/ml/mg protein). However, the ALA alone group showed an increase in SOD levels (1.57±0.17 µmol/ml/mg protein) when compared to the nicotine group (1.38 ± 0.11 µmol/ml/mg protein) (Figure 2).

GPx Activity
Figure 3 depicts the effect of ALA supplementation on GPx levels in the experimental rats. Nicotine reduced the GPx level in the treated rats (0.35±0.08 µmol/ml/mg protein) compared to the control (0.58±0.06 µmol/ml/mg protein; p<0.05). Administration of nicotine and ALA in another group resulted in a significant reduction (0.50±0.04 µmol/ml/mg protein) of the nicotine effect, thus raising GPx level towards normal (0.58±0.06 µmol/ml/mg protein).

Catalase Activity
Figure 4 shows the effect of alpha lipoic acid (ALA) supplementation on catalase activity in nicotine treated rats. In this study, there was no significant difference in catalase activity in all the experimental groups when compared to the control.

Table 1: Effect of Alpha Lipoic Acid Supplementation on Cardiovascular Parameters in Nicotine Treated Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Nicotine</th>
<th>Nicotine + ALA</th>
<th>ALA</th>
</tr>
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<tbody>
<tr>
<td>Systolic blood pressure (SBP) (mmHg)</td>
<td>109.6±8.61</td>
<td>144.05±4.80*</td>
<td>145.5±6.73*</td>
<td>116.4±3.47**</td>
</tr>
<tr>
<td>Diastolic blood pressure (DBP) (mmHg)</td>
<td>87.7±7.14</td>
<td>119.5±3.38*</td>
<td>116.1±6.30*</td>
<td>94.75±2.12**</td>
</tr>
<tr>
<td>Pulse pressure (PP) (mmHg)</td>
<td>21.85±2.38</td>
<td>24.55±3.86</td>
<td>29.48±2.33</td>
<td>21.68±3.69</td>
</tr>
<tr>
<td>Mean arterial blood pressure (MABP) (mmHg)</td>
<td>95.05±7.58</td>
<td>127.6±3.46*</td>
<td>125.8±6.35*</td>
<td>101.9±2.00**</td>
</tr>
<tr>
<td>Heart rate (HR) (beats/min)</td>
<td>430.8±18.5</td>
<td>437.0±8.11</td>
<td>409.8±11.16</td>
<td>405.1±7.17</td>
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<tr>
<td>Rate pressure product (RPP) (au)</td>
<td>47722.6±5244.1</td>
<td>62838.13±1715*</td>
<td>58949.3±3849.6*</td>
<td>47107.3±697.8**</td>
</tr>
</tbody>
</table>

* signifies significant difference from control (p<0.05), * signifies significant difference from nicotine (p<0.05), ** signifies significant difference from nicotine + ALA (p<0.05). Results are mean ± SEM (n = 7).
Antioxidant Effects of Alpha-Lipoic Acid

Fig. 1: Effect of Alpha Lipoic Acid (ALA) Supplementation on Malondialdehyde Levels in Nicotine Treated Rats.
* signifies significant difference from control (p<0.05), * signifies significant difference from nicotine (p<0.05). Results are mean ± SEM (n = 7).

Fig. 2: Effect of Alpha Lipoic Acid (ALA) Supplementation on Superoxide Dismutase Activity in Nicotine Treated Rats.
* signifies significant difference from control (p<0.05), # signifies significant difference from nicotine (p<0.05). Results are mean ± SEM (n = 7).

Fig. 4: Effect of Alpha Lipoic Acid (ALA) Supplementation on Catalase Activity in Nicotine Treated Rats.
There was no significant difference across the groups. Results are mean ± SEM (n = 7).

Lipid Profile: Total Cholesterol, Triglyceride, Low-Density Lipoprotein and High-Density Lipoprotein

Table 2 shows the effect of alpha lipoic acid (ALA) supplementation on lipid profile parameters in nicotine treated rats. There was no significant difference in serum cholesterol, triglyceride, and low-density lipoprotein across all groups when compared to control, nicotine and nicotine + ALA. However, in the ALA group, HDL levels were significantly increased compared to nicotine only and nicotine +ALA groups (p<0.05).

C-Reactive Protein (CRP)

Figure 5 shows the effect of ALA supplementation on C-reactive protein in nicotine treated rats. The levels of CRP were significantly increased (p<0.05) in the nicotine group.
(0.067±0.016 µg/ml) and nicotine + ALA group (0.063±0.012 µg/ml) when compared to the control group (0.038±0.005 µg/ml). However, there was a significant decrease in CRP levels in the ALA group (0.033±0.005 µg/ml) when compared to both the nicotine group and nicotine + ALA group.

![Fig. 5: Effect of Alpha Lipoic Acid (ALA) Supplementation on C-reactive Protein in Nicotine Treated Rats.](image)

* signifies significant difference from control (p<0.05), # signifies significant difference from nicotine (p<0.05). Results are mean ± SEM (n = 7).

DISCUSSION

Nicotine has been known to stimulate the sympathetic nervous system, consequently leading to increased myocardial work and a high risk of cardiovascular dysfunctions (6–8). The increased blood pressure and rate pressure products in the nicotine administered rats in this study are in line with various studies on blood pressure increase by nicotine (36–38). High blood pressure is a leading risk factor for non-communicable diseases such as stroke and heart diseases (1). It is responsible for 1.6 million deaths worldwide each year with 80 percent of those deaths occurring in low-and middle-income countries (1). Sympathetic stimulation of nicotine could be as a result of the direct effect of nicotine on the brain and autonomic ganglia or as a result of direct stimulation of the adrenal glands to release catecholamines (39–40), which further acts on the β-adrenergic receptor to cause increases in HR, BP and, myocardial contractility. Other studies have also indicated that the PP is a better predictor of arterial wall stress due to blood pressure; this increased stress leads to damaged blood vessels and ventricular hypertrophy (41–42). Results from this study showed that in rats administered nicotine and supplemented with ALA, the blood pressure values remained elevated, suggesting that ALA had no blood pressure lowering effects in the nicotine administered rats. However, since ALA has been known to reduce blood pressure in patients with diabetic nephropathy (43), this suggests that ALA does not affect the mechanistic pathway for which nicotine induced an increase in blood pressure.

Oxidative stress plays a key role in the development of many cardiovascular diseases, including atherosclerosis, hypertension, ischemia-reperfusion injury, and heart failure (18–19, 44). Studies have shown a link between oxidative stress in the brain and increased sympathetic activity and hypertension (26–28). Although the main mechanism of action of nicotine is by activating the sympathetic nervous system, oxidative stress plays a major role in nicotine toxicity. The current study showed an increase in MDA levels in nicotine administered rats and a corresponding decrease in the levels of the antioxidant enzymes. This confirms the presence of increased ROS in the nicotine administered rats; this result is in line with reports from several studies that confirm a change in antioxidant enzymes levels in nicotine administered rats and among smokers (12,45,46).

ALA has been shown to have favorable effects on cellular redox state and has been shown to decrease lipid peroxidation and cellular production of reactive oxygen species (21, 47). This study showed that ALA confers protection on the redox status of rats administered nicotine. MDA levels were decreased while SOD and GPx levels were increased. Glutathione peroxidase is considered one of the most important antioxidant agents involved in the protection of cell membranes from lipid peroxidation by scavenging oxygen radicals (48). ALA is considered a master antioxidant orchestrator, facilitating the optimal interactions among the other antioxidants. When ALA is missing, other antioxidants do not interact well, thereby reducing their ability to protect cells (49). ALA administration has been documented to increase intracellular glutathione levels by as much as 70% (50).

Altered lipid profile, inflammation, blood coagulation, impaired endothelial dysfunction are associated risk factors making smokers more susceptible to coronary heart disease than non-smokers (37, 51). Our study using nicotine found no difference in the lipid profile of nicotine administered rats compared to the controls. However, in rats administered ALA, HDL levels were significantly elevated compared to the other groups. This suggests that ALA could raise HDL level which is referred to as the “good cholesterol” that transports cholesterol from the cells and tissue back to the liver, preventing excess cholesterol from being deposited in the walls of the blood vessels. In models of diabetes with impaired lipid profile, ALA has been found to increase HDL levels (52, 53).

Elevation of CRP levels, a marker of inflammation, has been linked to atherosclerosis and heart disease (54–56). In this study, CRP levels were increased in nicotine treated rats suggesting that the administration of nicotine also produces inflammation. Co-administration of ALA and nicotine showed that ALA exerted no significant anti-inflammatory effects in rats treated with nicotine as CRP levels were still elevated.

CONCLUSION

This study reinforced that the primary mechanism by which ALA ameliorates nicotine-induced toxicities is via its antioxidant properties. Oxidative stress plays a key role in the development of many cardiovascular diseases. This study therefore, reveals a promising role for ALA to ameliorate nicotine-induced oxidative stress but not blood pressure indices in rats.
CONFLICT OF INTEREST
None declared by the authors.

REFERENCES


