FREE RADICAL SCAVENGING ACTIVITY OF Emblica officinalis DURING NICOTINE INDUCED TOXICITY IN RATS (Rattus norvegicus)

J. Vadivelu and S. Dawood Sharief*

School of Environmental Science, Post Graduate and Research Department of Zoology, The New College, Chennai-14, India.

Correspondence to: R. Dawood Sharief, School of Environmental Science, Post Graduate and Research Department of Zoology, The New College, Chennai-14, India.

E. mail: sdawoodsharief@yahoo.co.in

Received date: 12-12-13

Abstract

Emblica officinalis (Amla) is widely used in the Indian system of medicine and is believed to increase defense mechanism against diseases. It is one of oriental traditional medicine used for hepatic and lung disorders from time immemorial. Nicotine is the most abundant component in cigarette smoke; it is first metabolized in the liver and induced lung and liver damages. Present study was carried out to investigate the effect of Emblica officinalis on nicotine induced toxicity for antioxidant status in rats. Male wistar rats were used for the experimental study. Toxicity was induced by oral administration of nicotine at a dose of 5 mg/kg body weight for 32 days, in rats which caused a significant decrease in the levels of Superoxide Dismutase (SOD), Catalase (CAT), Total reduced glutathione (GSH) and Glutathione peroxidase (GPx) in plasma, lung, liver, kidney and brain. The levels of SOD, CAT, GSH and GPx were found to increase on administration of Emblica officinalis (250 & 500 mg/kg body weight) for 7 days (after 32 days of nicotine administration as maintained earlier). The result suggested that 500 mg/kg body weight group showed significance effect than the 250 mg/kg body weight indicating the effect to be dose dependent.

Key words: Emblica officinalis, Nicotine, Super oxide dismutase, Catalase and Glutathione peroxidase.

INTRODUCTION

Nicotine

Cigarette addiction, the most common form of tobacco product, continues to be one of the world’s most serious public health problems and it is responsible for large numbers of death worldwide even after media advertisement. The actions of nicotine have been extensively investigated in various tobacco product, continues to be one of the world’s most serious public health problems and it is responsible for large numbers of death worldwide even after media advertisement. The actions of nicotine have been extensively investigated in human, in animal, and in a variety of cell systems [1, 2]. It has been reported long back that it induces oxidative stress in both in vitro and in vivo [3, 4].

Oxidative stress is a disturbance in the pro-oxidant-antioxidant system leading to potential damage. This imbalance results in reactive oxygen species [5]. The resulting reactive oxygen species, which includes hydroxyl radicals, superoxide and hydrogen peroxide play an integral role in modulation of several physiological function but can also be destructive if produced in excessive amounts [6]. Nicotine is responsible for a high toxicity [7], the predominant effects of nicotine in the whole intact animal or human consist of an increase in pulse rate, blood pressure, plasma free fatty acids and lung injury [8, 9]. Free radical - induced oxidative damage has been suggested to play a major role in the pathogenesis of numerous smoking related disorders.

Nicotine, the major component of cigarette smoke plays an important role in the development of cardiovascular disease and cancer [10]. It also has been affects plasma level of Thyroid hormones and Corticosterone [11]. Nicotine has also been studied as an experimental therapy for Parkinson’s disease, Alzheimer’s disease, and ulcerative colitis [12, 13]. In addition, nicotine has also been found to disturb the antioxidant defense mechanisms in rats fed with a high fat diet [14, 15]. This can damage major cellular components including membrane lipids, protein, carbohydrates and DNA thus resulting in tissue damage [16]. Enhanced depletion of antioxidants in tissues during nicotine induced lung toxicity has also been reported by Sudheer et al. [17].

Emblica officinalis

Emblica officinalis (Phyllanthus emblica L.) is a euphorbiaceous plant widely distributed in subtropical and tropical areas of India, China, Indonesia, and Malaysia. It has abundant amounts of vitamin C and superoxide dismutase [18] and is used in many traditional systems of medicine. Many other countries add this as important dietary sources in addition to their use in traditional medicine for wound healing, inflammation and stomach acidity. Emblica fruit is reported to have hypolipidemic according to Anila and Vijayalakshmi [20] and found to be hypoglycemic [21].

Several investigators have determined the efficacy of amla as an anti-atherosclerotic [22] anti-diabetic [23] antimutagenic [24] and anticancer agents [25, 26]. It is also used as antimicrobial agent [27] and anti-inflammatory agent [28], antibacterial agent [29]. It was reported that emblica has a strong antioxidant activity [30, 31], which may be partially due...
to the existence of flavonoids and several gallic acid derivatives including epigallocatechin gallate [32, 33] which makes everyone to think Amla as a wonder fruit.

The aim of the present study was to investigate the effect *Emblica officinalis* on lipid peroxidation and antioxidant activity during nicotine induced toxicity in rats.

**MATERIALS AND METHODS**

**Animals**

Male albino rats (*Rattus norvegicus*) ranging in body weight from 175-200 gms were obtained from the King Institute, Guindy, Chennai and maintained according to the guidelines of CPCSEA (No: 324), under the supervision of Animal Ethical Committee were used for the experiment. They were acclimatized to laboratory conditions prior to use and fed with pelleted chow (supplied by Poultry Research Station, Chennai) and water provided *ad libitum*.

**Chemicals**

Nicotine (-) - nicotine ([1]-1methyl-2-[3-pyridyl]-pyrrolidine), was purchased from Sigma Fine chemicals, Chennai, India. Nicotine solution was prepared daily. (Seperate drinking bottles were used to avoid nicotine solution exposition to light).

**Plant material**

*Emblica officinalis* was procured from local market and fruit of *Emblica officinalis* was separated, shade dried, grounded with mortar and pestle and sieved to get fine powder.

**Experimental design**

The rats were randomly distributed into four different groups of six animals each under identical conditions and were grouped as follows:

- **Group -I** Served as control animals and was given drinking water.
- **Group- II** Animals received nicotine (5 mg/kg bwt) in drinking water for 32 days.
- **Group -III** Animals received *Emblica officinalis* (250 mg/ kg bwt) in drinking water for 7 days (after 32 days of nicotine administration).
- **Group -IV** Animals received *Emblica officinalis* (500 mg/ kg bwt) in drinking water for 7 days (after 32 days of nicotine administration).

At the end of the experimental period (40th day) all the animals were anesthetized and sacrificed by cervical dislocation after an overnight fast. Blood was collected and the serum and organs were separated for further studies.

**Antioxidant Estimation**

The activity of Superoxide Dismutase (SOD) [34], Catalase (CAT) [35], Total reduced glutathione (GSH) [36] and Glutathione peroxidase (GPx) [37] were assayed in plasma, lung, liver, kidney and brain on experimental animals.

**Statistical analysis**

The data were analyzed using Analysis of Variance (ANOVA) and the group means were compared by Duncan’s Multiple Range Test (DMRT). The difference was considered to be significant at p<0.05 level.

**RESULTS**

The activity of GSH, GPx, SOD, CAT, in plasma, lung, liver, kidney and brain were significantly decreased in nicotine treated animals when compare to the control animals. In these levels of GSH, GPx, SOD, CAT, for administration of *Emblica officinalis* group animals were decreased significantly when compared to the nicotine treated animals (Table 1- 4).

Significance protection was seen in the *Emblica officinalis* supplemented nicotine treated animals, but the 500 mg/kg body weight dose was more effective than the 250 mg/kg body weight dose tested.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Haemolysate U^a</th>
<th>Lung U^b</th>
<th>Liver U^b</th>
<th>Kidney U^b</th>
<th>Brain U^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.46 ± 0.26</td>
<td>11.65 ± 0.86</td>
<td>9.56 ± 0.64</td>
<td>7.94 ± 0.52</td>
<td>6.42 ± 0.62</td>
</tr>
<tr>
<td>Nicotine</td>
<td>2.48 ± 0.14</td>
<td>6.44 ± 0.42</td>
<td>13.38 ± 0.41</td>
<td>4.84 ± 0.32</td>
<td>3.68 ± 0.32</td>
</tr>
<tr>
<td>N + EO (250mg/bwt)</td>
<td>2.99 ± 0.36</td>
<td>8.86 ± 1.22</td>
<td>11.02 ± 0.78</td>
<td>5.68 ± 0.68</td>
<td>3.84 ± 0.72</td>
</tr>
<tr>
<td>N + EO (500mg/bwt)</td>
<td>3.24 ± 0.24</td>
<td>11.02 ± 0.81</td>
<td>9.38 ± 0.66</td>
<td>7.16 ± 0.51</td>
<td>6.03 ± 0.61</td>
</tr>
</tbody>
</table>

U^a Enzyme required for 50% inhibition of NBT reduction/min mg Hb; U^b Enzyme required for 50% inhibition of NBT reduction/min mg protein

Values not sharing a common superscript letter (a, b, c and d) differ significantly at P<0.05 (Duncan’s Multiple Range Test)

**Group comparison:** Group 1 with all; Group 3& 4 with 2.

**Table 2: Changes in the activity of CAT in plasma, lung, liver, kidney and brain (mean ± S.D.; n=6)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Haemolysate U</th>
<th>Lung U</th>
<th>Liver U</th>
<th>Kidney U</th>
<th>Brain U</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.02 ± 0.24</td>
<td>2.87 ± 0.86</td>
<td>72.65 ± 4.28</td>
<td>20.12 ± 1.24</td>
<td>4.86 ± 0.36</td>
</tr>
<tr>
<td>Nicotine</td>
<td>1.45 ± 0.13</td>
<td>7.64 ± 1.78</td>
<td>54.66 ± 2.61</td>
<td>10.86 ± 0.74</td>
<td>3.24 ± 0.28</td>
</tr>
<tr>
<td>N + EO (250mg/bwt)</td>
<td>2.24 ± 0.42</td>
<td>32.54 ± 2.87</td>
<td>62.42 ± 4.68</td>
<td>14.68 ± 1.68</td>
<td>3.82 ± 0.54</td>
</tr>
<tr>
<td>N + EO (500mg/bwt)</td>
<td>2.86 ± 0.22</td>
<td>73.52 ± 2.46</td>
<td>71.84 ± 4.16</td>
<td>19.24 ± 1.12</td>
<td>4.26 ± 0.34</td>
</tr>
</tbody>
</table>

U^a -μmoles of H_2O_2 utilized/min/mg Hb; U^b -μmoles of H_2O_2 utilized/min/mg protein.
Values not sharing a common superscript letter (a,b,c and d) differ significantly at P<0.05 (Duncan’s Multiple Range Test)

Group comparison: Group 1 with all; Group 3& 4 with 2.

Table 3: Changes in the activity of GPX in plasma, lung, liver, kidney and brain (mean ± S.D.; n=6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma (U/mg Hb)</th>
<th>Lung (U/mg tissue)</th>
<th>Liver (U/mg tissue)</th>
<th>Kidney (U/mg tissue)</th>
<th>Brain (U/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.46 ± 1.51</td>
<td>12.39 ± 0.84</td>
<td>9.86 ± 0.76</td>
<td>7.58 ± 0.57</td>
<td>3.86 ± 0.32</td>
</tr>
<tr>
<td>Nicotine</td>
<td>13.36 ± 0.89</td>
<td>7.32 ± 0.61</td>
<td>6.87 ± 0.46</td>
<td>4.87 ± 0.42</td>
<td>1.58 ± 0.14</td>
</tr>
<tr>
<td>N + EO (250mg/bwt)</td>
<td>20.24 ± 1.92</td>
<td>9.84 ± 0.98</td>
<td>7.05 ± 0.87</td>
<td>5.18 ± 0.69</td>
<td>2.04 ± 0.58</td>
</tr>
<tr>
<td>N + EO (500mg/bwt)</td>
<td>22.66 ± 1.48</td>
<td>11.36 ± 0.82</td>
<td>8.67 ± 0.56</td>
<td>6.89 ± 0.56</td>
<td>3.12 ± 0.34</td>
</tr>
</tbody>
</table>

U^A -µmoles of GSH utilized/min/mg Hb; U^B -µmoles of GSH utilized/min/mg protein.

Values not sharing a common superscript letter (a,b,c and d) differ significantly at P<0.05 (Duncan’s Multiple Range Test)

Group comparison: Group 1 with all; Group 3& 4 with 2.

Table 4: Changes in the activity of GSH in plasma, lung, liver, kidney and brain (mean ± S.D.; n=6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma (mg/dl)</th>
<th>Lung (mg/100 tissue)</th>
<th>Liver (mg/100 tissue)</th>
<th>Kidney (mg/100 tissue)</th>
<th>Brain (mg/100 tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32.42 ± 2.41</td>
<td>124.88 ± 7.38</td>
<td>136.65 ± 8.12</td>
<td>104.26 ± 6.26</td>
<td>35.25 ± 2.62</td>
</tr>
<tr>
<td>Nicotine</td>
<td>15.63 ± 1.62</td>
<td>56.76 ± 3.86</td>
<td>68.42 ± 5.26</td>
<td>168.35 ± 4.31</td>
<td>21.36 ± 1.89</td>
</tr>
<tr>
<td>N + EO (250mg/bwt)</td>
<td>27.64 ± 2.76</td>
<td>108.66 ± 7.86</td>
<td>116.21 ± 8.94</td>
<td>132.36 ± 6.78</td>
<td>28.42 ± 2.96</td>
</tr>
<tr>
<td>N + EO (500mg/bwt)</td>
<td>31.48 ± 2.38</td>
<td>122.36 ± 7.32</td>
<td>134.23 ± 8.06</td>
<td>107.23 ± 6.41</td>
<td>34.86 ± 2.59</td>
</tr>
</tbody>
</table>

Values not sharing a common superscript letter (a,b,c and d) differ significantly at P<0.05 (Duncan’s Multiple Range Test)

Group comparison: Group 1 with all; Group 3& 4 with 2.

DISCUSSION

Glutathione is a powerful tripeptide antioxidant that inhibits the formation of free radicals and is thought to be the most important cellular antioxidant [38]. As a substrate for the antioxidant enzyme, GPX, reduced glutathione protects cellular constituents from the damaging effects of peroxides formed in metabolism and other ROS reactions [39]. Decreased levels of GSH are associated with cell damage. GSH present in the lung lining plays a crucial role in protecting the lung from oxidative stress by detoxifying exogenous toxicants and quenching ROS [40]. GPX catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide and the reduction product of the hydroperoxides [41].

Enzymatic antioxidants like superoxide dismutase, catalase and glutathione peroxidase synergistically act against reactive oxygen species. SOD, CAT and GPX constitutes a mutually supportive team of defense against reactive oxygen species which have been found to be decreased in nicotine treated rats [42]. SOD is a ubiquitous enzyme with an essential function in protecting aerobic cells against oxidative stress, it is primarily a mitochondrial enzyme usually found in the plasma membrane [43]. Catalase is the tetrameric hemoprotein undergoes alternative divalent oxidation and reduction at its active site in the presence of H₂O₂ [44].

The hepatoprotective effect of *Emblica officinalis* extracts were related mostly to their reported antioxidant properties [45]. *Emblica officinalis* fruit extract neutralizes the oxidizing potentials of reactive oxygen species generated thereby maintaining cell membrane integrity and viability. Further the phytochemical analysis of the *Emblica officinalis* fruit revealed the presence of saponins, tannins, anthraquinones, coumarins, sterols and/or triterpenes. Moreover Oliver [46] in his study has reported that saponins, and tannins are known to affect the integrity of mucus membranes. Tannins also being stringent may have precipitated micro proteins on the site of ulcer thereby forming an impervious protective pellicle over the lining to prevent absorption of toxic substances and resist the attack of proteolytic enzymes [47]. Which may be cause in which our study shows that the administration of *Emblica officinalis* reversed these changes induced by nicotine in rats.

CONCLUSION

Thus the study shows the potent effect of *Emblica officinalis* as a very good antioxidant. Significant protection was seen in this study which proves the efficacy and effect of *Emblica officinalis* on nicotine induced toxicity in rats. Concentration of *Emblica officinalis* at 500mg/kg body weight was found be more effective in counteracting nicotine induced toxicity in rats showing a dose dependent response.

ACKNOWLEDGMENT

We take this opportunity to thank the Management, Principal & HOD of Zoology of The New College Chennai for facilities and encouragement.

REFERENCES


