

ORIGINAL RESEARCH ARTICLE

Role of *Emblica officinalis* (Amla) on Nicotine toxicity to rats (*Rattus orvegicus*)

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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 disorders from time immemorial. Nicotine is the most abundant component in cigarette smoke and it is first metabolized in the liver. The present study was carried out to investigate the role of *Emblica officinalis* on nicotine induced toxicity in rats. Animals were divided in to four group of with each group containing six rats. Male wistar rats (Group - II, Group - III and Group - IV) were treated with oral nicotine diluted with drinking water for 32 days, while (Group - I) control was administrated with drinking water simultaneously. After 32 days, Group - III and Group - IV were administered with two different concentrations of *Emblica officinalis* (250 mg/kg, 500 mg/kg body weight) for 7 days. Group - II served as a toxicity group (5 mg/kg body weight of nicotine). Rats were sacrificed 24 hours after last day of administration (40th day), the blood was analyzed for biochemical parameters.

A significance increase in the activity of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) and decreased the activity of Gamma Glutamyltransferase (γ -GT) was recorded, Total Protein and elevated levels of cholesterol, triglycerides in nicotine control group was observed. In group Group - III and Group - IV, the effect of *Emblica officinalis* was more significant in animals treated with 500 mg/kg dose. The results suggest that *Emblica officinalis* exerts the protective role of nicotine toxicity in rat.

Key words: *Emblica officinalis*, Nicotine, Alanine Aminotransferase, *Aspartate* Aminotransferase and Gamma Glutamyltransferase.

1. INTRODUCTION

Cigarette smoke has enormous negative health consequences worldwide, and the use of tobacco is still rising globally^[1]. Although, approximately 4000 components occur in the cigarette, nicotine is the alkaloid most active in the tobacco. Nicotine is an amine composed of pyridine and pyrrolidine rings^[2]. The actions of nicotine have been extensively investigated in human, in animal, and in a variety of cell systems^[3]. Nicotine is responsible for a high toxicity effect^[4], the predominant effects of nicotine in the whole intact animal or human increase the pulse rate, blood pressure, plasma free fatty acids and lung injury^[5, 6]. It has been reported long back that it induces oxidative stress in both *in vitro* and *in vivo*^[7].

In addition, nicotine has also been found to disturb the antioxidant defense mechanisms in rats fed with a high fat diet^[8, 9, 10]. It 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]. Nicotine is metabolized by various a pathway of which cotinine is the primary product of the C-oxidation pathway of nicotine biotransformation^[14], while the liver is considered to be the major site of nicotine biotransformation, metabolism also occurs in the lung and kidney^[15].

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^[16], and is used in many traditional medicinal systems. *Emblica* fruit is reported to have hypolipidemic^[17], hypoglycemic^[18] activities and also acts as an

important constituent of many hepatoprotective formulations available [19]. It is also used as antimicrobial [20], anticancer [21, 22] and anti-inflammatory agent [23]. It was reported that emblica has a strong antioxidant activity [24, 25], which may be partially due to the existence of flavonoids and several gallic acid derivatives including epigallocatechingallate [26]. The aim of the present study was to investigate the role *Emblca officinalis* on nicotine induced toxicity in rats.

2. MATERIALS AND METHODS

Animals

Male albino rats (*Rattus norvegicus* L.) ranging in body weight from 175 - 200 g 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. 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 ([-]-1methyl-2-[3-pyridyl]-pyrrolidine), was purchased from Sigma Fine chemicals, Chennai, India. Nicotine was prepared daily. (Special drinking bottles were used to avoid nicotine solution exposition to light).

Plant material

Emblca officinalis was procured from local market and fruit of *Emblca 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 clean drinking water.

Group - II Animals received nicotine (5 mg/kg b.wt) in drinking water for 32 days.

Group - III Animals received *Emblca officinalis* (250 mg/kg b.wt) in drinking water for 7 days (after 32 days of nicotine administration)

Group - IV Animals received *Emblca officinalis* (500mg/ kg b.wt) 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 anaesthetized and sacrificed by cervical dislocation after an overnight fast. Blood was collected and the serum was separated for further analysis.

Analysis of biochemical marker enzymes in the plasma

The activities of Alanine Aminotransferase (ALT) [27, 28], Aspartate Aminotransferase (AST) [29], Alkaline Phosphatase (ALP) [30, 31], Gamma Glutamyltransferase (γ -GT) [32] were assayed and the levels of total protein [33], Cholesterol [34], Triglycerides [35] were estimated in serum.

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.

3. RESULTS

The activities of enzymes in the plasma are shown in (Table 1). In nicotine treated rats, the activities of ALT, AST and ALP were significantly increased and γ -GT activity was decreased when compared with the control. Administration of *Emblca officinalis* to nicotine treated rats at two different doses significantly decreased the activities of enzymes such as ALT, AST and ALP showed increased activity of γ -GT when compared to the nicotine treated animals.

The levels of cholesterol, triglycerides and total protein in serum of experimental animals are shown in (Table 2). The levels of cholesterol and triglycerides in plasma of experimental animals increased and total protein level significantly decreased in the nicotine treated group when compared to the control. Significance protection was seen in the *Emblca officinalis* supplemented animals when compared with nicotine treated animals, but the 500 mg/kg body weight dose was more effective than the 250 mg/kg body weight dose tested.

Table 1: Changes in the activities biochemical marker of enzymes in the plasma [Values are mean \pm SD from 6 rats in each group]

GROUPS	ALT(U/L)	AST(U/L)	ALP(U/L)	γ -GT(U/L)
Control	38.18 \pm 2.46 ^a	73.16 \pm 4.32 ^a	78.34 \pm 3.26 ^a	8.3 \pm 0.38 ^a
Nicotine	76.21 \pm 4.62 ^b	134.24 \pm 12.61 ^b	164.20 \pm 8.42 ^b	3.4 \pm 0.32 ^b
N + EO (250mg/bwt)	43.12 \pm 3.12 ^c	77.21 \pm 4.26 ^c	90.18 \pm 4.61 ^c	6.4 \pm 1.27 ^c
N + EO (500mg/bwt)	39.32 \pm 3.26 ^d	74.18 \pm 3.68 ^d	80.04 \pm 6.48 ^d	7.6 \pm 0.23 ^d

N= nicotine; EO = *Emblcaofficinalis*; ALT= Alanine Aminotransferase; AST= Aspartate Aminotransferase; ALP= Alkaline Phosphatase; γ -GT= Gamma Glutamyltransferase.

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

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

Table 2: Changes in the level of cholesterol, triglycerides and total protein (Values are mean \pm SD from 6 rats in each group)

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Total Protein (g/dl)
Control	78.42 \pm 7.68 ^a	70.24 \pm 5.46 ^a	6.9 \pm 0.20 ^a
Nicotine	151.28 \pm 9.78 ^b	94.32 \pm 7.26 ^b	6.1 \pm 0.27 ^b
N + EO 250mg/bwt	84.32 \pm 6.72 ^c	73.67 \pm 6.48 ^c	6.3 \pm 0.33 ^c
N + EO 500mg/bwt	80.14 \pm 8.64 ^d	71.28 \pm 8.23 ^d	6.7 \pm 0.20 ^d

N= Nicotine; EO = *Emblca officinalis*.

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

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

4. DISCUSSION

Nicotine is the major component of cigarette smoking, which causes oxidative damage to liver, kidney, lungs, brain and heart; it is a potential oxidant, which is capable of producing free radicals and reactive oxygen species [36]. The nicotine induced free radicals react with bio membranes causing oxidative destruction of polyunsaturated fatty acids and forming cytotoxic aldehydes by lipid peroxidation [37]. Lipid peroxidation has been implicated in pathogenesis of a number of diseases [38]. An increase in the activity of marker enzymes like ALT, AST and ALP in nicotine treated rats indicates tissue damage and according to Wetscher *et al.* [39] this increase in the ALP activities is the indicative of cellular damage due to loss of functional integrity of cell membranes. Oxidative damage to the cellular membranes produces marked changes in molecular organization of lipids resulting in increased membrane permeability [40]. It may be noted that, Further lipid peroxidation can be used as an index for measuring the extent of damage that occurs in membranes of tissues as a result of free radical generation [41]. Lipid peroxidation was enhanced in liver of the nicotine treated rats. Nicotine is oxidized primarily in to its metabolite cotinine in liver, generates free radicals in tissues and induces oxidative tissue injury as stated by Wang, *et al.* [42]. Thus, the damage to the tissues in the nicotine treated animals may be due to the excessive generation of free radicals.

In the present study the cholesterol level was elevated in the nicotine treated animals. The prevalence of hypercholesterolemia and triglyceridemia has been reported in heavy smokers [43]. The increased level of cholesterol is attributed to the increased activity of 3-hydroxy-3-glutaryl CoA reductase (HMG-CoA reductase) and increased incorporation of labeled acetate in to cholesterol [44]. Nicotine decreased the activity of lipoprotein lipase resulting in elevated levels of triglycerides. Huttunen, *et al.* [45] envisages that this enzyme is involved in the uptake of circulating triglycerides rich lipoprotein

(chylomicrons or VLDL) by the extra hepatic tissues. Cryer [46] reported that the cells of adrenal medulla synthesize catecholamines by the stimulation of nicotine and adipose tissue lipolysis which is carried out by catacholamines, which in turn elevates the levels of cholesterols, triglycerides and also increases the fatty acids.

Total protein content is slightly decreased in nicotine treated rats that were found to recover with of the treatment with *Emblca officinalis*. This might be due to phytochemical compound present in the natural products (Amla) in reducing or detoxifying the effect of nicotine. According to Bandyopadhyay *et al.* [47], the total protein serves as a source of nutrition for the tissue and the liver function. The site specific oxidative damage of some of the susceptible amino acids is now regarded as the major cause of metabolic dysfunction during pathogenesis. Extensive liver injury may lead to decreased blood levels of total proteins synthesized exclusively by hepatocytes, which is reflected in this study on rats.

Emblca officinalis is rich in vitamin C and serves as good antioxidant [48]. The antioxidant properties of *Emblca officinalis* extract are many times more than that of water soluble vitamin E [49]. According to Antarkar *et al.* [50], *Emblca officinalis* is a constituent of various liver tonics used against acute viral hepatitis and other liver disorders. The hepatoprotective effect of *Emblca officinalis* extracts were related mostly to their reported antioxidant properties [51]. The phytochemical analysis of the *Emblca officinalis* fruit revealed the presence of saponins, tannins, anthraquinones, coumarins, sterols and/or triterpenes. From the literature listed, it shows that *Emblca officinalis* fruit extract neutralizes the oxidizing potentials of reactive oxygen species generated thereby maintaining cell membrane integrity and viability. Thus, the present study shows that *Emblca officinalis* serves as an effective scavenger of free radicals, which helps to protect the nicotine induced toxicity in rats, both biochemically in restoring the enzymes level and also helping/repairing the damages hepatocytes.

5. CONCLUSION

The present study proves the efficacy and protective role of *Emblca officinalis* on nicotine induced toxicity in rats. Concentration of *Emblca officinalis* at 500 mg/kg body weight was found to be more effective in counteracting nicotine induced toxicity in rats.

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