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Toxicity of Nicotine and the Role of *Emblica Officinalis* in Rats (*Rattus Norvegicus*)

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Abstract: ***Emblica officinalis (Amla) is widely used in the Indian system of medicine and is believed to increase defence mechanism against diseases. It is one of the oriental traditional medicine was 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 groups of which 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 serum was analyzed for kidney markers (urea & creatinine) and minerals (calcium and phosphorous). The rats treated with Nicotine showed a decreased in urea and creatinine, calcium and phosphorous content when compared with the control animals. On treatment with Emblica officinalis in 250 and 500 mg/kg body weight doses to rats showed a marked increase in urea and creatinine, calcium and phosphorous level when compared with the Nicotine treated rats. However, the higher dose (500 mg/kg) showed a pronounced effect when compared to 250 mg/kg body weight.***

Keywords: Nicotine, *Emblica officinalis,* Urea, Creatinine, Calcium and Phosphorous.

1. Introduction

Nicotine

Nicotine is a naturally occurring alkaloid found primarily in the members of the *solanoceou*s plant family, predominantly in tobacco plant (*Nicotiana tabacum*) (Wu *et al.,* 2002), and lower levels in other plants such as eggplant, tomato, potato, and green pepper, where it acts as a natural insecticide (Doolittle *et al.,* 1995). A large range of toxic effect of nicotine has been found in humans, as well as in experimental animals, and several targets have been susceptible to them (Dominoe *et al.,* 2004; Yildiz, 2004; Liu *et al.,* 2003). Physiological effects have been found in chronic cigarette smokers. In addition, nicotine has also been found to disturb the antioxidant defence mechanisms in rats (Kalpana *et al.,* 2005; Perlemuter *et al.,* 2005). Nicotine has also been studied as an experimental therapy for Parkinson's disease, Alzheimer's disease and ulcerative colitis (Baron, 1996; Birtwistle and Hall, 1996). Nicotine is metabolized by various pathways, of which cotinine is the primary product of the C-oxidation pathway of nicotine biotransformation (Wang *et al.,* 2005). While the liver is considered to be the major site of nicotine biotransformation, metabolism also occurs in the lung and kidney (Trushin and Hecht, 1999). The actions of nicotine have been extensively investigated in human, in animal, and in a variety of cell systems (Cooke and Bitterman, 2004; Valenca *et al.,* 2004). It has been reported long back that it induces oxidative stress both in vitro and in vivo (Church and Pryor, 1958). Nicotine is responsible for a high toxicity effect (Elli Slaughter *et al.,* 2012). 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 (Benowitz *et al.,* 2002; Liu *et al.,* 2001).

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 (Sani *et al.,* 2008; Verma and Gupta, 2004) 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 hypoglycemic activity (Abesundara *et al.,* 2004). Several investigators have determined the efficacy of amla as an anti- atherosclerotic (Thakur *et al.,* 1988), antidiabetic (Tripathi *et al.,* 1979), antimutagenic (Agrawal *et al.,* 2012) and anticancer agents (Zhang *et al.,* 2004). It is also used as antimicrobial agent (Rani and Khullar, 2004) and anti-inflammatory agent (Perianayagam *et al.,* 2004), antibacterial agent (Saeed and Tariq 2007). It was reported that *Emblica* has a strong antioxidant activity (Islam *et al.,* 2008; Bafna and Balaraman, 2004), which may be partially due to the existence of flavonoids and several gallic acid derivatives including epigallocatachin gallate (Anila and Vijayalakshmi, 2002; Sabu and Kuttan, 2002). And also contain Vitamin C, minerals and amino acids. (Zhang *et al.,* 2000).

Emblica officinalis is known for its antioxidant properties and for its therapeutic effects, and is a component in more than hundred herbal formulations that are widely used in India and other countries. The fruits of *Emblica* are widely consumed raw, cooked or pickled, but they are also principle constituents of Ayurvedic preparations (Scartezzini *et al.,* 2006). In Unani medicine, it is described as a tonic for heart and brain. The fruits of (Amla) are used in many medicinal preparations of Ayurvedic and Unani systems of medicine (Kritikar and Basu, 1933). They are used in the treatment of leucorrhea and atherosclerosis (Jeena and Kuttan, 1995). Amla is also used for the treatment of various gastric ailments including dyspepsia (Kapoor, 1990).

2. 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 ([-]-1methyl-2-[3-pyridyl] - pyrrolidine), was purchased from Sigma Fine chemicals, Chennai, India. Nicotine solution was prepared daily. (Separate 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 b.wt) in drinking water for 32 days.

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

Group -IV Animals received *Emblica officinalis* (500 mg/ 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 and organs were separated for further studies. The Urea content was estimated as per Tiffany *et al.,* (1972) and Creatinine by Seation and Ali (1984), Calcium by Clark and Collip (1925), Kannan and Ravindranath (1980), Phosphorous by Friedman and Young (1997).

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

Table 1: Changes in kidney markers (Urea and Creatinine) in rats (*Rattus norvegicus*) treated with Nicotine and *Emblica officinalis*

Parameters	**Urea** (mg/dl)	**Creatinine** (mg/dl)
Control	38 ± 2.29 [a]	1.16 ± 0.12 [a]
Nicotine (5 mg/kg)	23 ± 1.62 [b]	0.64 ± 0.06 [b]
N+EO (250 mg/kg)	31 ± 2.26 [c]	0.78 ± 0.06 [c]
N+EO (500 mg/kg)	38 ± 2.37 [d]	1.24 ± 0.12 [d]

N-Nicotine; EO-*Emblica officinalis*.
Values represent mean ± SD of six animals

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

The effect of Nicotine toxicity and treatment with Emblica *officinalis* on kidney markers (Urea and Creatinine) in rats (*Rattus norvegicus*) is shown in table 1.The rats treated with Nicotine showed a decrease in urea and creatinine content when compared with the control animals and this decrease was statistically significant at P<0.05 level. On treatment with *Emblica officinalis* in 250 and 500 mg/kg body weight doses to rats showed a marked increase in urea and creatinine level when compared with the Nicotine treated rats and this increase was statistically significant at P<0.05 level and reached the value close to the control rats.

Table 2: Changes in Minerals (Calcium and Phosphorous) in rats (*Rattus norvegicus*) treated with Nicotine and *Emblica officinalis*

Parameters	Calcium (mg/dl)	Phosphorus (mg/dl)
Control	9.12 ± 0.86 [a]	6.4 ± 0.64 [a]
Nicotine (5 mg/kg)	6.81 ± 0.67 [b]	4.8 ± 0.44 [b]
N+EO (250 mg/kg)	7.54 ± 0.68 [c]	5.7 ± 0.56 [c]
N+EO (500 mg/kg)	9.32 ± 0.80 [d]	6.5 ± 0.62 [d]

N-Nicotine; EO-*Emblica officinalis*.
Values represent mean ± SD of six animals
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

The effect of Nicotine toxicity and treatment with *Emblica officinalis* on minerals (Calcium and Phosphorous) in rats (*Rattus norvegicus*) is shown in table 2. The Nicotine treated rats showed a decrease in calcium and phosphorous content when compared with the control animals and the decrease was statistically significant at P<0.05 level. While treating with Emblica *officinalis* with 250 and 500 mg/kg body weight doses to rats, they showed a increase in calcium and phosphorous level when compared with the nicotine treated animals and the increase was statistically significant at P<0.05 level.

4. Discussion

Nicotine is the primary component in cigarette smoke that alters lung development, leading to decreased pulmonary function (Sekhon *et al.,* 1999; Sekhon *et al.,* 2001; Sekhon *et al.,* 2004). Although there are numerous studies describing the effects of maternal smoking on lung function in children and adults, most of them are unable to discern the exact period of exposure that is most relevant (Upton, 2004). Smoking is a major risk factor for at least 20 diseases, including coronary and peripheral vascular disease, chronic bronchitis and at least 80% of lung cancers (Callum, 1998). Although nicotine itself is not carcinogenic, tobacco smoke contains over 200 other compounds that are potential carcinogens and smoking itself is the greatest single risk factor for lung cancer. Cigarette smoking significantly contributes to several other nasopharyngeal and upper

gastrointestinal carcinomas (Benowitz, 1988). There are reports that certain plants and herbs have defence mechanisms for Nicotine and in this have study we have selected *Emblica officinalis*. It is "one of the best rejuvenating herbs" in the Ayurveda. The fruits of the plant are also recommended as a rasayana, which promotes health and longevity by increasing defence against diseases, arresting the aging process and revitalizing the body in debilitated conditions (Satyavati *et al.,* 1976).

Emblica officinalis is used for the treatment of liver disorders, indigestion, stomach ulcers, diabetes, inflammatory diseases and inhibition of tumor growth and in geriatric complaints. It also functions as potent antioxidant agent (Ghosal *et al.,* 1996). Pozharitskaya *et al.,* (2007) have demonstrated that *Emblica officinalis* extract contains several antioxidants such as emblicanin A and B, gallic acid, ellagic acid, ascorbic acid that possesses strong antioxidative activity. Amla is an important dietary source of minerals, amino acids, tannin and sugar. It protects against radiation (Scartezzini, and Speroni, 2000); possess antidiabetic activity (Sabu and Kuttan, 2002), cytoprotective and immunomodulatory (Sai *et al.,* 2002).

Urea is non-protein nitrogenous product whose normal level in blood reflects continued protein metabolism. Creatinine levels serves as a marker of kidney function and alterations in the levels of urea and creatinine indicate the disturbed renal function. Present study revealed decreased levels of urea and creatinine in nicotine exposed rats, while on treatment *Emblica officinalis*, the same increased and attained near normal values depending upon the dosage which is in accordance with reports of Iranloye and Bolarinwa (2009). As nicotine toxicity was also affect in serum mineral levels. Low levels of calcium and phosphorus were found in nicotine toxicity rats which may be due to liver cell damage (Tietz, 1976). The decrease in calcium and phosphorous might be due to nicotine toxicity damaging the liver that could have utilized the available calcium and phosphorous in turn reducing the protein content which was seen in the present study. This argument goes along with the studies of Tietz (1976) who observed that hypocalcaemia is generally associated hypoproteinemia.

The Urea and Creatinine, Calcium and Phosphorous level recovered from the toxicity when *Emblica officinalis* was administered after 7 days. However, the higher dose (500 mg/kg) showed a pronounced effect when compared to 250 mg/kg body weight. This suggested that the recuperation of all the cell counts might be due to the synergic action of other phytochemicals present in *Emblica officinalis*.

5. Conclusion

The present study was, on effect of nicotine which causes alteration in kidney markers (Urea & Creatinine), minerals (Calcium & Phosphorous). Using *Emblica officinalis* an antitodes to combat toxicity due to nicotine, the results were satisfactory indicating the role of *Emblica officinalis*, since *Emblica officinalis* contains vitamin C, gallic acid, flavonoids, minerals, tannins, alkaloids, phenolic compounds, amino acids and carbohydrates, etc. which can detoxify the effect of Nicotine on haematological parameters. The constitution of *Emblica officinalis* might be the causative factors as modifiers in the changed metabolism due to exposure of nicotine toxicity.

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