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PROTECTIVE EFFECT OF EMBLICA OFFICINALIS ON NICOTINE INDUCED TOXICITY IN RAT BLOOD (RATTUS NORVEGICUS)

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Abstract:

This study was designed to observe the protective effect of Emblica officinalis in nicotine toxicity on various haematological parameters of adult male rat. 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 was analyzed for haematological parameters. Nicotine toxicity on rats showed the parameters such as White Blood Cell (WBC), Red Blood Cell (RBC), Haemoglobin (Hb), Packed Cell Volume (PCV) and platelet count were found to decrease in all the parameters tested, whereas Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) showed a significant increase respectively when compared with the blood parameters of control animals. Treatment with Emblica officinalis (250 and 500 mg/kg body weight of dose), showed a remarkable recovery from the Nicotine toxicity when compared with control animal. On treatment with Emblica officinalis in 500 mg/kg body weight dose shows more effective than 250 mg/kg body weight dose, when compared with the Nicotine treated animals.

Key words: Nicotine, Emblica officinalis, White Blood Cell , Red Blood Cell ,Haemoglobin, Packed Cell Volume, platelet, Mean Corpuscular Volume (MCV).

1. INTRODUCTION

Nicotine

Nicotine is a naturally occurring alkaloid found primarily in the members of the solanoceous 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 defense 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).

Emblica officinalis

Emblica officinalis is a medium to large deciduous tree belonging to a small sub genus of trees of the Euphorbiaceae widely growing in different parts of India, Srilanka, Pakistan, Uzbekistan and China. 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). The wide use of Emblica officinalis in India for various purposes prompted us to select the same to treat Nicotine toxicity which has not been standard so far. Under these circumstances Emblica officinalis opens up a new avenue to treat Nicotine related problems. The fruit Emblica officinalis Gaertn., syn: Phyllanthus Emblica (Euphorbiaceae), Emblic myrobalan locally known as Amla or Amlaj is one of the important herbal drugs used in Unani (Graeco-Arab) and Ayurvedic systems of medicine. It is used both as a medicine and as a tonic to build up lost vitality and vigor. 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). The fruits are acrid, cooling, refrigerant and diuretic. They are useful in hemorrhage, diarrhea and dysentery. Amla fruits are anabolic, antibacterial and resistance building (Charaka, 1941). They possess expectorant, cardiotonic, antipyretic, antioxidative, antiviral and antiemetic activities. 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* 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 pelletted 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

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 clean 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* (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 for further analysis.

Analysis of Haematological changes

The white blood cell (WBC) count was determined by the method of Raghuramulu et al (1981), the red blood cell (RBC) count was determined by diluting a measured quantity of blood with fluid isotonic solution as per the method of Huxtable (1990), Haemoglobin (Hb) was determined by Cyanomet Haemoglobin method of Drabkin and Austin (1932), Packed cell volume (PCV) was determined by centrifugation using Wintrobe tubes according to method of Samuel (1980), Using haemoglobin, packed cell volume (PCV) or hematocrit and red blood cell count values we can determine MCV, MCH, MCHC values, by using standard formulas.

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. RESULT

Haematological changes

Parameters	WBC (10^3/µl)	RBC (10^6µl)	Hb (g/dl)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dl)	Platelet (10^5/mm^3)
Control	12.46 ± 1.24 [a]	7.61 ± 0.74 [a]	13.26 ± 1.34 [a]	41.32 ± 2.26 [a]	48.72 ± 2.16 [a]	16.81 ± 1.06 [a]	30.42 ± 1.64 [a]	7.46 ± 0.68 [a]
Nicotine (5 mg/kg)	6.83 ± 0.56 [b]	4.23 ± 0.37 [b]	8.42 ± 0.66 [b]	26.43 ± 1.64 [b]	54.61 ± 3.42 [b]	19.32 ± 1.02 [b]	32.61 ± 1.57 [b]	5.34 ± 0.54 [b]
N+EO (250 mg/kg)	9.32 ± 1.05 [c]	5.98 ± 0.48 [c]	9.71 ± 1.02 [c]	34.55 ± 2.16 [c]	51.78 ± 3.23 [c]	14.98 ± 1.21 [c]	27.24 ± 1.64 [c]	6.93 ± 0.63 [c]
N+EO (500 mg/kg)	11.78 ± 1.06 [d]	6.98 ± 0.56 [d]	12.31 ± 1.21 [d]	40.02 ± 3.06 [d]	48.63 ± 3.29 [d]	16.41 ± 1.34 [d]	30.82 ± 1.55 [d]	7.38 ± 0.65 [d]

Table.1. Changes in haematological parameters in rats (*Rattus norvegicus)* treated with Nicotine and *Emblica officinalis*

Nicotine; AA-Ascorbic acid; WBC- White blood cells; RBC – Red blood cells; Hb-Haemoglobin; PCV-Packed cell volume
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 for 32 days followed by treatment with *Emblica officinalis* for 7 days on haemotological parameters in rats (*Rattus norvegicus)* are shown in table 1.The parameters such as WBC, RBC, Hb, PCV and platelet count were found to decrease in all the parameters tested and this decrease was statistically significant at P<0.05 level, whereas MCV, MCH and MCHC showed a significant increase and this increase was statistically significant at P<0.05 level respectively when compared with the blood parameters of control animals. Treatment with *Emblica officinalis* (250 and 500 mg/kg body weight of dose), showed a remarkable recovery from the Nicotine toxicity and the changes after treatment with *Emblica officinalis* are statistically significant at P<0.05 level for the above said parameters in a dose dependent pattern when compared with the Nicotine toxicity rats, but it very clear that when 500 mg/kg body weight of *Emblica officinalis* was administered there was significantly change in all the blood parameters and the changes were statistically significant at P<0.05 level when compared with control animals.

4. DISCUSSION

Heamatological changes, due to administration of nicotine brought out decrease in WBC, RBC, Hb, PCV, platelet count and a slight increase in MCV, MCH, MCHC levels. Similar reports were observed by (Metin *et al.*, 2004). According to them, due to consumption of smokeless tobacco, the total blood WBC, RBC, monocytes and platelets counts decreased, which is in aggrement with the previous study, while Murat *et al.*, (2006) reported that Maras powder consumers (smokeless tobacco) have increased MCV, MCH and MCHC and decreased WBC, RBC, Hb. This fact was corroborated in our study with rats. Blood coagulates in smokers more easily than in non-smokers, fibrinogen levels are higher and platelets are more likely to aggregate. These effects all contribute to thromboembolic diseases. Haematological parameters 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

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 deaths worldwide. Conservative estimates suggest that by 2020, up to 10 million people will die worldwide annually due to tobacco related diseases, because a large range of toxic compound

present in the Nicotine. Plants and plant products are widely used as agents for the prevention and cure of many diseases. Plants are generally considered to be less toxic and free from many of the side effects than synthetic drugs. The present study was, on effect of nicotine which causes alteration in haematological parameters. 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.

REFERENCES

1. Baron, J. A. Beneficial effects of nicotine and cigarette smoking: the real, the possible and the spurious. *Br. Med. Bull.,52*:58-73, 1996.
2. Birtwistle, J. & Hall, K. Does nicotine have beneficial effects in the treatment of certain diseases. *Br. J. Nurs., 5*: 195- 202, 1996.
3. Charaka SC (1941) Nirnaya Sagar Press, Bombay, India, pp 114–115.
4. Chruch, D.F. and W.A. Pryor, 1958. Free radical chemistry of cigarette smoke and its toxicological implications. *Enviro. Health Perspect*. 64: 111-126.
5. Cooke, J. P. & Bitterman, H. Nicotine and angiogenesis: a new paradigm for tobacco-related diseases. *Ann. Med., 36*:33-40, 2004.
6. Dominoe, E.F., Ni, L., Xu, Y., Koeppe, R.A., Guthrie, S., Zubieta, J-K., 2004.
7. Doolitle, D.J., Winegar, R., Lee, J.K., Caldwell, W.S., Wallace, A., Hayest, J., Bethlzy, J.D., 1995. The genotoxic potential of nicotine and its major metabolites. *Mutat. Res.* 344, 95–102.
8. Drabkin, D.L and Austin, J.H. 1932. Spectrophotometric studies, spectrophotometric constants for common hemoglobin derivatives in human, dog and rabbit blood. *J. Biol. Chem.* 98: 719-733.
9. Huxtable, A.I 1990, In: Hand book of Endotoxin. Proctor, R.A. (ed), *Elsevier Amsterdam.*
10. Jeena KJ, Kuttan R (1995) Antioxidant activity of *Emblica officinalis.* Journal of Clinical Biochemistry and Nutrition19: 63- 70
11. Kalpana, C.; Rajasekharan, K. N. & Menon, V. P. Modulatory effects of curcumin and curcumin analog on circulatory lipid profiles during nicotine-induced toxicity in Wistar rats. *J. Med. Food, 8*:246-50, 2005.
12. Kapoor LD (1990) CRC Handbook of Ayurvedic Medicinal Plants. CRC Press, Inc. Boca Raton. Florida. 175-176.
13. Kirtikar KR, Basu BD (1933) *Indian medicinal plants*. Basu Ltd, Allahabad, p 488.
14. Metin K, Erdogar O, Lihami Y, Fatma I, Ergul BKS. 2004. The investigation of the effect of Maras's powder (smokeless tobacco) on hematological parameters 21(3): 131-136.
15. Murat A, Hasan CE, Mustafa C, Ciragil P, Mustafa G. 2006. Comparison of effects of smoking and smokeless tobacco "Maras Powder" use on Humoral immune system Parameters. (14): 1-4.
16. Perlemuter, G.; Davit-Spraul, A.; Cosson, C.; Conti, M.; Bigorgne, A.; Paradis, V.; Corre, M. P.; Prat, L.; Kuoch, V.; Basdevant, A.; Pelletier, G.; Oppert, J. M. & Buffet, C. Increase in liver

antioxidant enzyme activities in non-alcoholic fatty liver disease.*Liver Int., 25*:946-53, 2005.

17. Raghuramulu, N., Nair, K.M and Kalyansundaram, S. 1981. In: Hand book of Endotoxin. Proctor, R.A. (ed), *Elsevier Amsterdam.*
18. Regional cerebral blood flow and plasma nicotine after smoking tobacco cigarettes. *Prog. Neuro-Psychopharmacol. Biol. Psych*. 28, 319–327.
19. Samuel, K. M. 1980. Notes on clinical laboratory techniques. Silver Prints, Hydrabad, pp. 254.
20. Scartezzini P, Antognoni F, Raggi MA, Poli F, Sabbioni C (2006). Vitamin C content and antioxidant activity of the fruit and of the Ayurvedic preparation *Emblica officinalis* Gaertn. J. Ethnopharmacol. 104: 113-118.
21. Trushin, N. & Hecht, S. S. Stereo selective metabolism of nicotine and tobacco- specific N-nitrosamines to 4- hydroxy-4-(3- pyridyl) butanoic acid in rats. *Chem.Res.Toxicol., 12*:164-71, 1999.
22. Valenca, S. S.; de Souza da Fonseca, A.; da Hora, K.; Santos, R. & Porto, L. C. Lung morphometry and MMP-12 expression in rats treated with intraperitoneal nicotine. *Exp. Toxicol. Pathol., 55*:393-400, 2004.
23. Wang, S. L.; He, X. Y. & Hong, J. Y. Human cytochrome p450 2s1: lack of activity in the metabolic activation of several cigarette smoke carcinogens and in the metabolism of nicotine. *Drug Metab.Dispos., 33*:336- 40, 2005.
24. Wu W, Ashley DL, Watson CH (2002). Determination of nicotine and other minor alkaloids in international cigarettes by solid-phase microextraction and gas chromatography/mass spectrometry. Analytical Chemistry 74, 4878–4884.
25. Yildiz, D., 2004. Nicotine, its metabolism and an overview of its biological effects. Toxicon 43, 619–632.