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ACUTE INHALATION OF PASSIVE SMOKING ON LUNG OF ALBINO RAT AND AMELIORATIVE ROLE OF AMLA *(PHYLLANTHUS EMBLICA)*

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ABSTRACT

In the present study, alteration due to passive smoking of beedi and supplementation of aqueous extract of amla has been observed in BAL fluid in albino rats. The experiment was conducted on 15 albino rats with weighing 90-120g. The albino rats were divided into 3 groups randomly A, B & C. Group A consist of 5 albino rats which are unexposed. Group B consist 5 albino rats which are exposed to passive smoking of 4-beedi for 1hour per day with time interval of 5 min for 28 days. Group C also consist of 5 albino rats which are exposed to passive smoking of 4-beedi along with supplementation of aqueous extract of amla (200 mg/kg b.wt.) for 28 days. BAL fluid counts declined after supplementation of aqueous extract of amla in comparison to beedi smoke exposed rats of both the sexes due to antioxidant defense mechanism.

KEY WORDS: BAL Fluid, (TLC& DLC), Passive smoke of beedi, Amla extract, Albino rats.

INTRODUCTION

Environmental pollution is a worldwide problem, made by human activity and by natural forces as well (Fereidoun *et al*., 2006). Smoking is also a source of air pollution and cause severe damage to the health. Smoking is produced by burning of leaves and absorbed into blood stream. Tobacco smoke is also an important source of indoor air pollution, contributing to a noxious environment, eye irritation and unpleasant odour. Passive smoking is the involuntary inhaling of smoke from other people's cigarette, cigar, beedi, etc. Breathing in other people's smoke, also called as second hand smoke.There is increasing evidence that passive smoking is an important factor in chronic respiratory disease.

Smoking greatly affects our lung and airways. Smokers get a variety of problems related to breathing. Problems range from an annoying cough to grave illness like emphysema and cancer. When we smoke beedi, many chemicals enter our body through our lungs. Burning tobacco produces more than 4000 chemicals (Rodgman and Perfetti, 2009). Tobacco smoking, now ranks as the third most common cause of death in the United States and the fifth worldwide. This condition is initiated by respiratory exposure to noxious particles or gas, most commonly tobacco smoke, which triggers an inflammatory response in the lung. Beedi smoke is recognized as a crucial factor in the development and pathogenesis of chronic obstructive pulmonary disease (COPD).

Amla (phyllanthus emblica) is one of the plants that exhibit various therapeutic properties. The amla fruits are reported to contain thermostable vitamin-C, minerals, amino acids, tannis, flavonoids and other important phytochemicals which are believed to possess divers pharmacological and biological effects (Feeney, 2004). It is also reported to be free radical scavenging, anti-diabetic, antihyperlipidemic, anti-inflammatory, antiatherosclerotic, antimutagenic, chemopreventive and hepatoprotective (Santoshkumar *et al.*, 2013).

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However, protection from the impact of beedi smoke on mammals still awaits further investigation.

Therefore, the present study is carried out to assess the possible amieliorative role of amla in relation to BAL fluid counts viz : Total Leukocyte Count (TLC) and Differential Leukocyte Count (DLC) of beedi smoke exposed rats. The albino rats, *Rattus norvegicus* (Brekenhout) have been taken for the present study due to its easy to handle in the laboratory conditions and similarity to the human beings physiologically.

MATERIALS AND METHODS

Healthy and adult albino rats were taken for the present study. The albino rats (80-150g) were kept in a polypropylene cages measuring 45cm × 27cm × 15cm at the temperature 25±0 °C and relative humidity 50±5% and 12 hrs/day light-dark cycle. The roof for cages made up of galvanized steel mesh. A sliding removable tray was placed below the cage to hold excreta which was cleaned regularly to avoid any kind of infection and undesirable odour in the laboratory. The cages were well equipped with a metal food plate and water bottle. The rats were fed with balanced food rich in all stuffs necessary to maintain their health before and during exposure and water was provided *ad libitum*. Cages were cleaned regularly to avoid any infection and odour in laboratory. There are three sets (A, B & C) of albino rats (each set contain 5 albino rats).

Experimental protocol

Control set-A : Unexposed rats

Experimental set-B : Exposed to passive smoking of four beedi smoke for one hour per day with time interval of 5 min for 28 days.

Experimental set-C : Exposed to passive smoking of four beedi smoke with supplementation of aqueous extract of amla (200 mg/kg b. wt.) for one hour per day with time interval of 5min for 28 days.

Collection of Brancheoalveolar Lavage Fluid (BALF)

The Brancheoalveolar lavage fluid was collected from the lung of albino rat by puncturing the trachea with the help of sterilized disposable syringe with 18 swg hypodermic needle. The lung was washed with 2 mL normal saline (20%) by injecting in lung and withdrawing it which contains the cells of Brancheoalveolar lavage. This procedure was repeated five times. The withdrawing fluid was pooled in a sterilized plain vial and this pooled fluid was used for Brancheoalveolar lavage fluid study.

Total and differential leukocyte counts of inflammatory cells in Brancheoalveolar lavage fluid (BALF) of albino rats were observed.

Total Leukocyte Counts

TLC was examined by new improved Neubourhaemocytometer (Dacie and Lewis, 1968)

Statistical analysis

Mean and standard deviation for each exposure groups were calculated and analysed by student 't' test computed by statistical software Pac version 3.0.

RESULTS

BAL fluid counts altration in control and exposure days are summarized in Table 1.

Exposed and control groups are averaged and analysed by student 't' test.

Table 1. TLC (×10^3/mL) & DLC (%) in BAL fluid of albino rats after exposure to passive smoking along with supplementation of aqueous extract of amla

Parametres	Control (5) Mean±S.Em.	Experimental Set-B	Experimental Set-C
TLC (×10^3/mL)	7.9-8.9 (8.5±0.17)	10.2-13.6 (12.5±0.61)↑**	8.2-9.6 (9.0±0.24) ↓**
Neutrophilcounts (%)	20-35 (27.8±2.90)	15-20 (16.6±1.07)↓**	10-30 (20.6±3.17) ↑*
Macrophage counts (%)	45-60 (51.6±2.5)	50-70 (61±1.28) ↑**	35-55 (42.6±3.50) ↓**
Lymphocyte counts (%)	0-3 (1.8±0.58)	3-6 (4.4±0.50) ↑**	0-4 (2±0.70) ↓**

DISCUSSION

There is a biological plausibility that passive smoking affects the pulmonary function and BAL fluid similar to the effect of active smoking. Passive smoking causes indirect changes in lung function in albino rats, while decrease and increase in BAL fluid counts approximately to their normal ranges have been observed after the supplementation of aqueous extract of amla in the beedi smoke exposed rats.

In the present study, a significant increase in Total Leukocyte Count (TLC), lymphocyte and macrophages. While, decrease neutrophil count are observed in BAL fluid of albino rats after inhalation of passive smoking. Alteration in the Total leukocyte count (TLC) and Differential leukocyte count (DLC) of BAL fluid is the sign of irritation which causes pulmonary inflammation in albino rats. An elevation in Total leukocyte count (TLC) of BAL fluid is the migration of various leukocytes of blood from the capillary accumulate at the site of inflammation (Casio *et al*., 2002) also reported that smoke induced the lower number of neutophils in mice will be lead to less neutrophil elastase. (Rennard *et al*., 2006) have reported that there is enhanced recruitment of inflammatory cells to the lung, partially neutrophil and macrophages in response to second hand smoke (SHS). (Macnee, 2007) reported that there is the several fold increase in the number of macrophages and dendritic cells in the airways of smokers and smoke exposed animals in comparison to controls. (Bazerra *et al*., 2011) studied that long term smoke exposure in mice lead to significantly increased inflammation, as measure by the infux of alveolar macrophages, lymphocytes and antioxidant enzymes (Karimi *et al*., 2012) also reported that smokers have an increased Total leukocyte count (TLC) mainly due to increase in macrophages in BAL fluid.

Present study suggests that the oral administration of aqueous extract of amla has a potential to diminish toxic effects induced by beedi smoking in albino rats. There is an improvement in Total leukocyte counts (TLC) and Differential leukocyte count (DLC) in BAL fluid of albino rats. Amla is rich in vitamins-C and serves as good antioxidant, anti-inflammatory, chemopreventive, immunomodulatory, and antimutagenic. The phytochemical analysis of amla fruit reveal the presence of flavanoids, phenolic and alkaloids. Ascorbic acid (vitamin-C), which is abundantly present in amla fruit, has the potential to shelter the body from oxidative cascades leading to inflammatory injury. (Dougan *et al*., 2011) noted that long term use of anti-inflammatory agents have been linked to decreased cancer incidence, indicating inflammation as a contributor to cancer development (Yokozawa *et al*., 2017) also observed that *Emblica officinalis* extract has been reported to inhibit NF-8^{o}B activation, a key transcription factor involved in chronic inflammatory response and ageing.

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