



Alterations in some haematological parameters in cigarette smoke exposed albino rat with supplementation of omega-3 fatty acids

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Abstract

Many compounds formed by cigarette burning, ranging from particulate materials to water solutes and gaseous extracts, are considered to be noxious agents, and many biochemical and molecular mechanism have been proposed for toxic effects of cigarette smoke. Smoking and its complications are of the most important social and health problems in all countries. High stress jobs favour repeated smoking and further rain force addictive behaviours. It is increasing to a large extent from school going children to old age person and even women are also taking interest in cigarette smoking. Some natural antioxidants alter these toxic effects to some extent. Keeping these points in view, the present study is designed to observe the effect of cigarette smoke with ameliorative effect of omega-3-fatty acids.

Keywords: cigarette smoke, omega-3-fatty acids, TEC, TLC, haemoglobin concentration

Introduction

Cigarette smoke is complex mixture of chemicals produced by burning tobacco and additives. The smoke contains for, which is made up of more than 4,000 chemicals and that at least 60 of these chemicals are identified as those which can cause cancer. Some compounds found in cigarette smoke including nicotine, carbon monoxide, hydrogen cyanide, ammonia, formaldehyde, benzene, methanol, acetylene and tar. All these substance are harmful for lung cancer, cardiovascular and many respiratory diseases. Cigarette smoke has been implicated as a major risk factor in chronic obstructive pulmonary diseases such as chronic bronchitis and emphysema, in carcinogenesis, and in cardiovascular disease. Cigarette smoking accounts for at least 30% all cancer deaths (Kalra *et al.*, 1994) [6].

The smoke of cigarette is rapidly absorbed into blood stream, increase the rate of heart beat and blood pressure because of the stimulation of the nervous system by the nicotine. Cigarette smoking like wire thickens the blood, thus increasing the risk of blood clots. Blood helps in various metabolic activities of the body and defend the body from immunologic reactions, and bleeding and maintain body temperature (Tarazi *et al.*, 2007) [13]. Any change in the composition of the blood can affect the body metabolism. Antioxidants are free-radical “Scavenger” and help in preventing the cell damage. Omega-3 fatty acids can be found in fish and certain plant oil. It is a polyunsaturated fatty acids (PUFAs) contains three major types of omega-3 fatty acids i.e. alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Hardman *et al.*, 2002) [5]. Omega-3 fatty acids can help in reducing the toxic effects of cigarette smoke on blood tissue.

The albino rat will be selected for the present study because of its easily adaptability and having physiological similarity to human beings and can be easily reared in laboratory conditions. The present study is, therefore, designed to assess the effect of cigarette smoking inhalation and role of antioxidant on some haematological parameter of albino rats, *Rattus norvegicus* (Berkenhout).

Materials and Methods

Experimental animal and maintenance

The wistar albino rat, *Rattus norvegicus* (Berkenhout) of both the sexes have been selected for the present study. The colony of wistar albino rats was bred at the animal house of Zoology Department, Dr. B.R. Ambedkar University, Agra. Healthy and adult albino rats of both the sexes of almost equal size and weight ranging from 100-158g were taken for the present study. They were kept in polypropylene cages measuring 45x27x15cms at temperature 21±5°C, relative humidity 60±5% and photoperiod 12hrs/day. The top of the cages was made of galvanized steel mesh. A sliding removable tray was placed below the cages to hold excreta which was cleaned regularly to avoid any undesirable odour in the laboratory. Each cage was equipped with a metallic food plate

and a water bottle. The rats were fed on Gold Mohar rat and mice feed, manufactured by Hindustan Unilever Ltd., India and water was provided *ad libitum*. The experimental albino rats were acclimated for one month prior to experiment.

Cigarette smoke

Cavanders Gold Leaf non-filtered cigarette, Godfrey Phillips India Ltd., Chakala, Andheri East, Mumbai was selected for the present study. A non-filtered cigarette requires 920mg tobacco and tar yields in the cigarette smoke is 22.1mg. The average nicotine yields of the cigarette smoke at that time are around 2.8mg (NCI, 1980).

Antioxidant

Omega-3 fatty acids were used as an antioxidant in the present study. Omega-3 fatty acids are a family of unsaturated fatty acids that have in common a carbon-carbon double bond in the n-3 position: that is the third bond from the methyl end of the fatty acid. Mega-3 gelatin capsules from Dr. Reddy's Laboratories Ltd., Hyderabad was used as an antioxidant. Each gelatin capsule contain 300mg Omega-3 fatty acids (180mg EPA and 120mg DHA). 30mg/100g body weight Omega-3 fatty acids were given to each rat/day.

Experimental protocol and exposure

The albino rats were grouped in three sets (1, 2 and 3). Each set contains five rats. The control set-1 is exposed to ambient air for one hour per day for 28days. Experimental set-2 is exposed to cigarette smoke one hour per day for 28days. Experimental set-3 is exposed to cigarette smoke with pre-exposure supplementation of Omega-3 fatty acids for one hour per day for 28days. For the exposure to cigarette smoke the experimental rats were kept in an isolated smoke chamber within their cages. The rats were subjected to whole body exposure of cigarette smoke of a non-filter cigarette 1hr/day (delivered as 3 cigarettes, 3 times/day) for 28days, at the bottom of smoke chamber by slow suction. The rats of control and experimental sets were exposed according to the experimental protocol (*vide supra*) for one hour per day for 28days.

Haematological studies

The rats of control set (1) and experimental sets (2 and 3) were sacrificed after 28 days for haematological study. The double oxalate vials were used for various haematological parameters. 800 mg potassium oxalate and 1200 mg ammonium oxalate were dissolved in 100 ml distilled water. After shaking well, 0.1 ml/ml of blood were taken in each sterilized empty vial and dried at 80°C in an oven. The blood samples were taken directly from the ventricles of the dissected rats with the help of 5.0 ml sterilized disposable syringe, filled with (22 SWG) hypodermic needles and blood was transferred immediately into double oxalate vials (*vide supra*) for various haematological parameters. The haemoglobin concentration was estimated by the standard Sahli's method (Wintrobe *et al.*, 1981) ^[15]. Total RBC count were estimated with the help of improved Standard Neubauer Haematocytometer (Dacie and Lewis, 1969) ^[2]. The total WBC count was made by using Standard Neubauer Haematocytometer (Dacie and Lewis, 1969) ^[2]. The results are subjected to statistical analysis through computer software based on Fisher and Yates (1963) ^[3].

Results and Discussion

The results show decrease in total RBC count and haemoglobin concentration after cigarette smoke exposure, while increase in total WBC count. The toxic effect ameliorated by omega-3-fatty acid as shown in Table 1-3 and Figure 1-3.

Table 1: The values of Total RBC count ($\times 10^{12}/L$) after cigarette smoke exposure and supplementation with Omega-3 fatty acids in albino rats

Sets	Treatment	Treatment days	Range (Mean \pm S.Em)	Significant difference from corresponding	
				Set-1	Set-2
Control set-1 (5)	Ambient air	28 days	6.34-8.25 (7.32 \pm 0.35)	-	-
Experimental sets Set-2 (5)	Cigarette smoke	28 days	5.34-7.05 (6.32 \pm 0.36)	P<0.05 \downarrow **	-
Set-3 (5)	Cigarette smoke + Omega-3-fatty acids	28 days	7.02-7.89 (7.311 \pm 0.17)	P>0.05 \downarrow *	P<0.05 \uparrow **

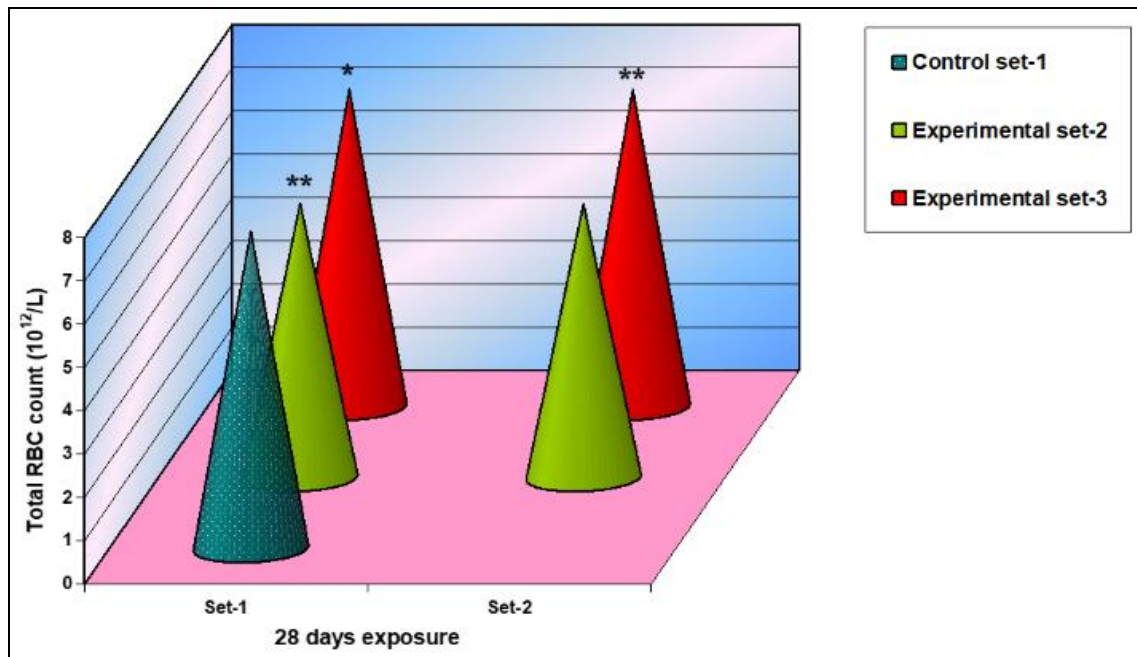


Fig 1

Table 2: The values of Haemoglobin concentration (g/dl) after cigarette smoke exposure and supplementation with Omega-3 fatty acids in albino rats

Sets	Treatment	Treatment days	Range (Mean±S.Em)	Significant difference from corresponding	
				Set-1	Set-2
Control set-1 (5)	Ambient air	28 days	15.33-16.60 (15.83±0.22)	-	-
Experimental sets Set-2 (5)	Cigarette smoke	28 days	12.09-14.25 (13.36±0.48)	P<0.01↓***	-
Set-3 (5)	Cigarette smoke + Omega-3-fatty acids	28 days	15.25-16.09 (15.85±0.16)	P>0.05↑*	P<0.01↑***

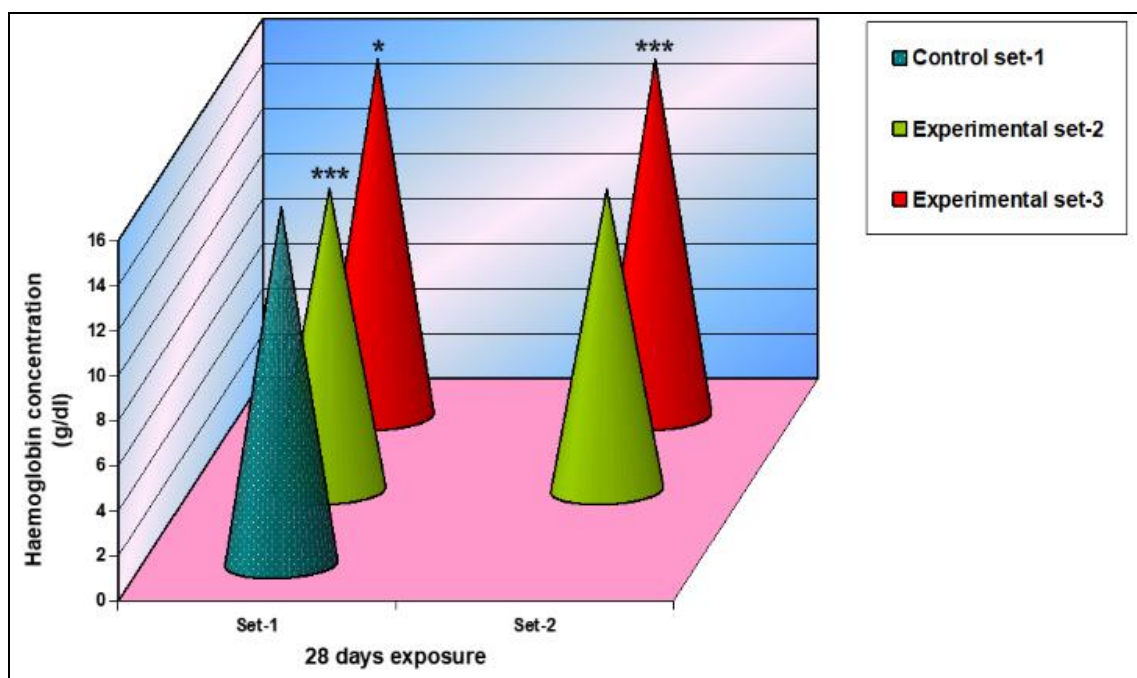


Fig 2

Table 3: The values of Total WBC count ($\times 10^9/L$) after cigarette smoke exposure and supplementation with Omega-3 fatty acids in albino rats

Sets	Treatment	Treatment days	Range (Mean \pm S.Em)	Significant difference from corresponding	
				Set-1	Set-2
Control set-1 (5)	Ambient air	28 days	8.0-9.0 (8.45 \pm 0.21)	-	-
Experimental sets Set-2 (5)	Cigarette smoke	28 days	9.5-10.6 (9.96 \pm 0.196)	P<0.01 \uparrow ***	-
Set-3 (5)	Cigarette smoke + Omega-3-fatty acids	28 days	9.2-9.6 (9.12 \pm 0.190)	P<0.05 \uparrow **	P<0.05 \downarrow **

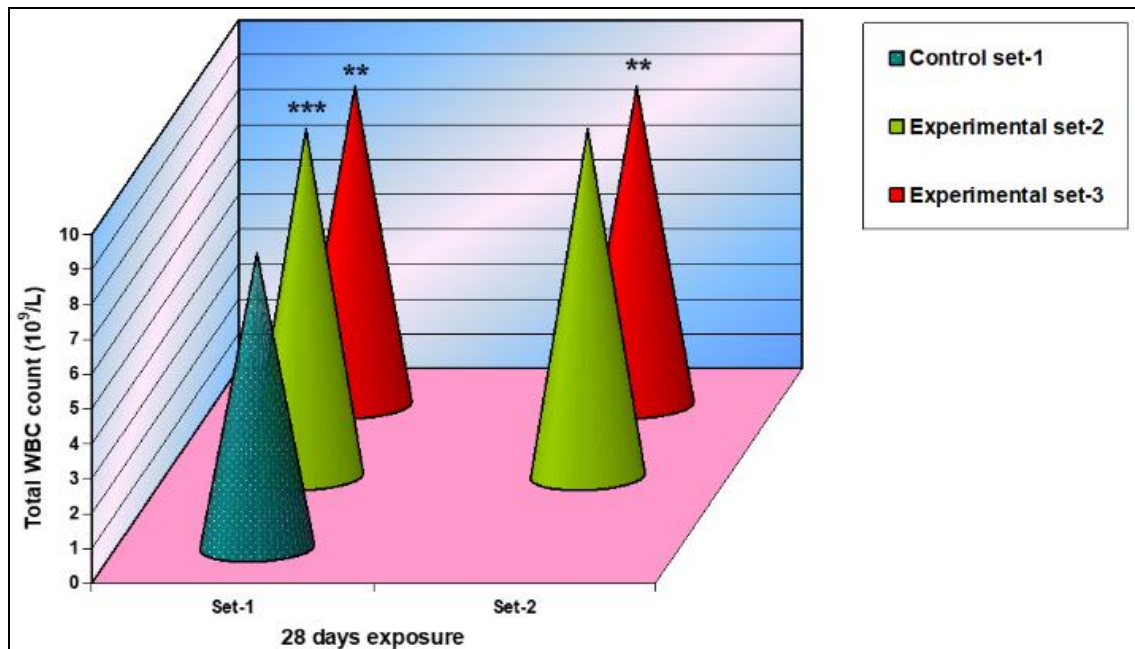
S. Em = Standard Error of Mean

** - Significant (P<0.05)

 \uparrow - Increase

(5) = No. of rats

*** - Highly significant (P<0.01)

 \downarrow - Decrease**Fig 3**

A significant reduction in total RBC count and haemoglobin concentration is correlated with the toxic effects of smoke on haemopoietic system. Cigarette smoke contains a toxic gas, carbon monoxide which binds to haemoglobin in red blood cell and interfere with body's ability to transport and utilized oxygen. The carbon monoxide in tobacco smoke exerts. Its negative effect by reducing oxygen carry capacity and leads to hypoxic anaemia in albino rats. Hypoxia is a major factor involved in tissue injury. In agreement with the present findings, Sasikala *et al.* (2003) ^[11] have reported a decrease in total RBC count and haemoglobin concentration in human after inhalation of cigarette smoke. Ludviksdottir *et al.* (2007) ^[7] have also reported a decrease in haemoglobin concentration during smoking in subjects.

In the present study, a significant increase in the total WBC count is result of inflammation due to irritated action of cigarette smoke on respiratory tract in albino rat, which causes leukocytosis. The upper respiratory tract represent the first contact area for smoke compound. Nicotine on the other hand can disturbed the function of adrenal gland under the smoke induced stress condition. In the agreement with the present findings, Tiel (2003) ^[14] have reported an increased total WBC count due to effect of cigarette smoking in human. Sasikala *et al.* (2003) ^[11] have also observed an increase in total WBC count after active and passive cigarette smoking. Further, Moszczynski *et al.* (1989) ^[8] have reported that the suppression of the haemopoiesis by the cigarette smoke result remarkable changes in peripheral blood picture. Ames (1989) ^[1] have also stated that reactive oxygen species

(ROS) can produced by cigarette smoke-induced phagocytic cells and causes oxidative damage. Gulshba *et al.* (2020) ^[4] reported acute inhalation of passive smoking on lung of albino rat and ameliorative role of amla (*Phyllanthus emblica*), while Syed and Shangloo (2020) ^[12] reported effect of nicotine smoke on liver and kidneys of adult male albino rats and Pedro *et al.* (2020) ^[10] reported protective Effects of Quercetin on Livers from Mice Exposed to Long-Term Cigarette Smoke.

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