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CIGARETTE SMOKE INTENSITY AND AN AMELIORATIVE ROLE OF VITAMIN C ON BLOOD CELL COUNTS IN ALBINO RATS

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ABSTRACT

In the present study, cigarette smoke exposure shows significant alterations on blood parameters viz., Total Erythrocyte counts (TEC), Haemoglobin concentration (Hb.con.), packed cell volume (PCV), Erythrocyte Sedimentation rate (ESR), Total leukocyte counts and platelets count in albino rats. These changes may be due to the oxidative stress and inflammatory responses induced by cigarette smoke on haematopoietic system. Cigarette smoke consists of carbon monoxide and nicotinamide, which are toxic gases having the capacity to binds with the haemoglobin in red blood cells and interferes with the body's ability to supply inadequate oxygen to the tissues that acts as a stimulus for marrow production in the result of hypoxic polycythemia and also induced an irritated action of tobacco smoke on the respiratory tract which causes leukocytosis in albino rats. While after supplementation of antioxidant vitamin C, which reduces the toxicity generated by cigarette smoke to a maximum extent. This is due to the antioxidant defence mechanism of vitamin C, which mitigates the toxic actionof cigarette smoke in albino rats.

KEY WORDS: Cigarette smoke, Vitamin C, Blood cells, Albino rats

INTRODUCTION

The burning of tobaccoleaves produces smoke which is absorbed into the bloodstream. The smoke of tobacco is also a source of indoor air pollution, causing eye irritation and unpleasant odour. Cigarette smoking is associated with a high social standing value in comparison to beedi smoke. Consumption of tobacco is through smoking is the most common and regular method. The smoke of cigarettes consists of a complex mixture of chemicals produced by the burning of tobacco and its additives, also contain tar, which is the build-up of more than 4000 chemicals. The smoke of cigarettes generates several toxic compounds like nicotine, ammonia, acrolein, phenols, acetaldehyde, carbon monoxide, hydrogen cyanide, nitrogen oxides and free radical. Cigarette smoking is linked with increased oxidative stress and produced an inflammatory stimulus on macrophages of the lung to produce many more free radicals; these play a role in the development of chronic bronchitis, inflammatory diseases and emphysema. Approximately 1014 free radicals and 1015 oxidant radicals are present in a single puff of cigarette smoke distributed between gases and particles (Pyror, 1997). These include different compounds which have the ability to causing an increase in the generation of several reaction oxygen species (ROS). Antioxidants play an important role in scavenging of free oxygen radicals and settled the cell membrane, and maintain its permeability. Cigarette smoking is very common in our environment in which most of us live, induces the production of (ROS) in the tissues. ROS are the free radicals thatare generated spontaneously in living cells during several metabolic pathways. An imbalance between the generation of ROS and a biological system has the ability to detoxify readily. The reactive intermediates cause oxidative stress and inflammatory responses. To prevent free radicals damage body has an elaborate antioxidant defence

system.

Tobacco consumption is increasing 3.4% per year in developing countries, and according to a WHO report, one ten adults is killed with cigarette smoking-associated diseases. Cigarette smoke is injurious to both smokers and non-smokers exposed to second-hand smoking; it is the type of smoke that fills every enclosed space when people burn tobacco products such as cigars, beedi, cigarettes and water pipes (WHO, 2012). The main three components of tobacco smoke are tar, carbon monoxide and nicotine. Each cigarette contains about 10 milligram of nicotine which is a very powerful, highly additive stimulant drug. The average smoker takes in 1-2 milligrams of nicotine per cigarette. When tobacco is smoked, nicotine rapidly reaches peak levels in the bloodstream and enters the brain. Tar is a complex mixture of substances, which in sufficient quantities are thought capable of initiating and promoting cancer. The smoke of tobacco is a complex mixture containing more than 4500 different constituent, including nicotine and carbon monoxide are the chief components of tobacco smoke these are mixed with the bloodstream and distributed in the entire body.

MATERIALS AND METHODS

Fifteen adult wistar albino rats (100-150 g) of both sexes were used in the present study. The animals were kept in polypropylene cages at the temperature 25±0.5 °C, relative humidity 60± 5% and a photoperiod of 12 hr/day. Each and every cage was equipped with a metal food plate and a plastic water bottle. The cages were cleaned regularly to avoid any kind of infection and undesirable odour in the laboratory. The rats were fed on Gulmohar rat, and mice feed (Hindustan lever Limited, India) water was given *ad libitum.*

Selection of cigarettes: Godfrey Capstan, Pilot Philips, India ltd., Kolkata, India was taken, or the present work 69 cm was the length of a cigarette.Antioxidants vitamin C (Ascorbic acid) is water soluble.Cecon drops manufactured by Pharmacia health care company Navi Mumbai was used, an antioxidants vitamin C (5 mg) was given to each rat per day.

Experimental protocol: The albino rats of both sexes were divided into three sets containing five rats each. Set I: control (unexposed); Set II: Exposed to cigarette smoke one hour per day for four weeks; Set III: Exposed to cigarette smoke with pre-exposure supplementation of vitamin C (5 mg/rat) for one hour per day for four weeks. Experimental rats were exposed in smoke chamber, manufactured by Precision Instrument, Varanasi with their cages. The rats were subjected to whole-body exposure of six filtered cigarettes/hour/day for four weeks.

Haematological studies: Rats of control set (I) and experimental sets (II) and (III) were sacrificed after four weeks. Blood samples were collected directly from the ventricles of the heart of the dissected rat with the help of sterilized disposable syringe fitted with hypodermic needles and were taken into double oxalate vials. Various haematological parameters were analysed by the following procedure in control and experimental rats.

Total Erythrocyte Count was estimated with the help of improved standard Neubauer Hematocytometer (Dacie, *et al*., 1996). The Haemoglobin concentration was done by the standard Sahli's method (1982). Erythrocyte Sedimentation Rate was determined by the Wintrobe's method (1982). Packed cell volume was calculated by the Wintrobe's method (1982). Total Leukocyte Count was estimated with the help of improved standard Neubauer Hematocytometer (Dacie, *et al*., 1996). Platelet Count was done with the help of improved standard Neubauer Hematocytometer (Dacie, *et al*., 1996).

The results of the present study were expressed as 5 Mean+S.em. by using student 't' test and statistical analysiswere done by one way ANOVA with the help of computer statistical program Kpkyplot (version 3.0).

RESULTS AND DISCUSSION

The values of haematological parameters in control set (I) and experimental sets (II and III) forfour week exposure are given in Table 1. In the present study the alterations in the values of haematological parameters viz., Total Erythrocyte Counts (TEC), Haemoglobin Concentration (Hb.conc.), Packed Cell Volume (PCV), Erythrocyte Sedimentation rate (ESR), Total leucocytes counts (TLC) and Platelet counts (PLT) is correlated with the oxidative stress and inflammatory action due to increase in carbonmonoxide concentration in the blood of cigarette smoke exposed albino rats. Carbon monoxide and nicotinamide are the chief components of tobacco smoke. These two induces the inadequate supply of oxygen to the tissues that act as a stimulus for marrow production is the

Table 1. Values of TEC, Hb. Conc., PCV, ESR, TLC and after cigarette smoke exposure and supplementation with vitamin C in albino rats. S.Em. = Standard Error of Mean, (P<0.05)—Significant**, (P<0.01) Highly Significant***, ↑-Increase↓ - Decreas, (P<0.001)-Very Highly Significant*** (5) = No. of Test animals.

Set	Exposure	Time Period	TEC ($x10^6$/µl) Mean±S.Em.	Hb.Conc. (g/dl) Mean±S.Em.	PCV (%) Mean±S.Em.	ESR (mm/hr) Mean±S.Em.	TLC ($x10^3$/µl) Mean±S.Em.	PLT COUNT ($x10^3$/µl) Mean±S.Em.
Control Set-I (5)	Ambient air	4 weeks	7.30± 0.27	12.0± 0.38	44.7± 0.77	4.1± 0.65	8.6± 0.26	892± 14.30
Experimental Set-II (5)	Cigarette smoke	4 weeks	8.19± 0.28***↑	13.9± 0.39***↑	42.1± 0.29**↓	2.7± 0.17**↓	9.45± 0.42***↑	628± 20.18***↓
Experimental Set-III (5)	Cigarette smoke + Vitamin C	4 weeks	7.01± 0.13**↓	11.8± 0.16**↓	46.4± 0.88**↑	3.8± 0.37**↑	8.41± 0.52**↓	802± 12.6***↑

outcome of hypoxic polycythemia and leukocytosis in albino rats. But after supplementation of antioxidant (vitamin C) these changes (*vide -supra*) becomes reduced to an extent.

Cigarette smoking can cause an elevation in red blood cells, haemoglobin concentration and white blood cells while, a decrease in the Packed Cell Volume (PCV), Erythrocyte Sedimentation rate (ESR), and Platelet counts (PLT) is due the oxidative stress and inflammatory responses produced by free radical generation in albino rats.

Similar to the present findings, Pederson *et al.* (2019) also reported an elevation in white blood cells and red blood cells after exposure to cigarette smoke in adult smokers. Malenica *et al.* (2017) have observed Cigarette smoking induced a significant increase in red blood cells, white blood cells, haemoglobin concentration. While a decrease in erythrocyte sedimentation rate, packed cell volume and platelet count in male and female workers. Sherke *et al.* (2016) have also stated that an increase in white blood cells, red blood cells, haemoglobin conc. While a decrease in erythrocyte counts, packed cell volume and platelet count by the effect of cigarette smoking induced inflammatory responses in male smokers. Nadia *et al.* (2015) observed a higher level in white blood cells, red blood cells and haemoglobin concentration after exposure to cigarette smoke in smokers.

In the present study, after exposure to cigarette smoke an increase in Total Erythrocyte counts, Hb. conc. while, a decrease in Erythrocyte sedimentation rate and Packed cell volume have been reported in the albino rats. Similar to the present findings, Kumar *et al.* (2012) who stated that increased value of haemoglobin and Red blood cells in smoker of male and female Gitte (2012) has reported that high level of haemoglobin and red blood cells in cigarette smokers. Abbas *et al.* (2019) have examined the higher value of haemoglobin and a red blood cell in cigarette smokers. Tiel *et al.* (2002) has observed an elevation in packed cell volume in smokers. Oseni *et al*. (2006) have also stated that Haematocrit value is directly linked with red blood cells and haemoglobin concentration in male smokers. In the support of present findings, Kume *et al.* (2009) have stated that increase in the red blood cells and haemoglobin concentration in male and female smokers.

In the present study, an increase in the total leukocyte counts is the outcome of inflammation due to irritated action of tobacco smoke on respiratory tract which causes leukocytosis. Upper respiratory tract represents the first contact area for smoke components. Al-Awadhi *et al*. (2008) have observed that leucocytosis occurs due to inflammation induced by smoking. Kume *et al.* (2009) have found that cigarette smoke generated reactive oxygen species leading elevation in white blood cells in male female smokers. Haider, *et al.* (2010) also reported an increase in white blood cell counts due to cigarette smoke in humans. Chang *et al*. (2010) and Watanabe *et al.* (2011) have also stated that nicotine induced the increase level of White cell counts in male smokers.

In the present study, adecrease in the platelet count is associated with the decrement in platelet production by the bone marrow after exposure to cigarette smoke. Dysfunction of platelet count occurs due to the presence of carbon monoxide in cigarette smoke which inhibits the platelet release. Rajshekhar *et al*. (2007) observed a decrease in platelet count after smoking in volunteers. Butkiewicz *et al.* (2006) also reported the slow platelet count due to cigarette smoker exposure in smokers.

To mitigate the toxic effect of cigarette smoke supplementation of vitamin c has been given to albino rats. Vitamin C is a water soluble vitamin that reacts with peroxy radical formed in the cytoplasm before they reach the cell membrane (1994) and serves to regenerate the reduced vitamin E (Takano *et al*., 1983). It is also essential for wound healing proper adrenal function, the formation of collagen, tissue health, the absorption of others nutrients, and control bleeding and maintain cellular oxygen turnover andcell membranes. Vitamin c also protects against oxidative stress and inflammatory disorders.

CONCLUSION

Our findings, demonstrated that continuous cigarette smoking has adverse effects on haematological parameters (Total Erythrocyte counts (TEC), Haemoglobin concentration (Hb.con.), Packed cell volume (PCV), Erythrocyte Sedimentation rate (ESR), Total leukocyte counts and platelets count in albino rats) and these alterations might be associated with the condition of oxidative stress and inflammatory responses resultant in form of, leucokytosis and polycythemia. To compensate these supplementation of antioxidants (vitamin C) has given to the rats parallel with cigarette smoke to maintain the body's imbalance.

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