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ORIGINAL ARTICLE

Protective Effect of Dietary Antioxidant Carotenoid on Blood Parameters in Albino Rats after Combined Exposure of SO_2 and NO_2

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ABSTRACT

In the present study, significant alterations in hematology of albino rats were observed after combined exposure to sulphur dioxide and nitrogen dioxide gas. The toxic effects induced by combined gases were mitigated after supplementation of dietary antioxidant carotenoids to an extensive limit. Both air pollutants sulphur dioxide and nitrogen dioxide produce their toxicity through free radical reactions in the biological system. Nitrogen dioxide gas dissolves in blood in the form of NO_2^- and NO_3^-, while sulphur dioxide can be converted into a mixture of sulphite, bisulphite and sulphur trioxide. Although the cells are protected from these reactive oxygen system by a number of cellular defence mechanism. Moreover, the carotenoids are the important physiological molecules that contribute to the natural antioxidant defence in biological system.

Key words: *Antioxidant Carotenoid , Blood Parameters, Albino Rats*

INTRODUCTION

Unlimited exploitation of nature by man which has disturbed the delicate ecological balance of nature. Various chemical substances are released in environment either by natural activities like volcanic eruptions or human activities such as burning of fossil fuels in industries, automobiles and agriculture activities as, are the form of pollutants. The pollutants may be solid, liquid or gaseous form. These pollutants change air quality and cause hazards to life. The common air pollutants are oxides of sulphur and nitrogen, carbon dioxide, carbon monoxide, ammonia, hydrocarbons and particulate matter etc. Sulphur dioxide is colourless gas with penetrating and pungent smell. It is highly soluble gas and consequently is absorbed in the moist passage of the upper respiratory system leading to airway resistance and stimulated mucus secretion, while nitrogen dioxide is deep reddish brown poisonous gas with characteristic smell. It is a chief constituent of photochemical smog and absorber of ultraviolet radiations. It accumulates in the atmosphere. Nitrogen dioxide is also absorbed in moist passage of upper respiratory system. High concentration of both nitrogen dioxide and sulphur dioxide gas is able to transport deep in the lung from where these gases enter the blood stream. Free radicals arise from sources both inside (endogenous) and outside (exogenous) our body. Exogenous free radicals formed from environmental factors such as pollution, exercise, sunlight, smoking, alcohol and X-ray. Antioxidants are nutrients (vitamin and minerals) as well as enzymes (protein) in our body that assist in chemical reactions. They are believed to play a role in preventing the development of such chronic diseases as cancer, heart disease, stroke and cataracts. The role of antioxidants is to neutralize free radicals. Antioxidant molecules have the ability to lose electrons without forming a chain reaction. Antioxidants react easily with oxygen and protect the other neighbouring cells from damage. They quench free radical and promote healthy cells. Antioxidants are found in our food in the forms of vitamin A, C, E and lycopene. These antioxidants work individually or in combination. Blood is an index of the state of health of organism and fluid constituent of the body that flows through vascular channels and transports the vital requirement and waste products of the body. Red blood cells and white blood cells perform function of transportation of oxygen and provide defense to body respectively. After respiratory system, next phase of contact, blood is the closest tissue to the inhaled NO_2 and SO_2. Thus, effect on blood tissue caused by inhaled gases might be more pronounced than on the other tissue, except respiratory apparatus. Any change in the blood tissue affects other tissues and

organs because of special function of blood (Guleria and Agarwal, 2017). Recent data from various agencies like Central Pollution Control Board, Uttar Pradesh Pollution Control Board show that concentration of sulphur dioxide is less than nitrogen dioxide in Agra (CPCB, 2006). Thus for present investigation, concentration of sulphur dioxide is taken half that of concentration of nitrogen dioxide. The haematological parameters of clinical interest are chosen for present study to know about the deleterious combined effects of sulphur dioxide and nitrogen dioxide on haematology and protective role of various antioxidants.

MATERIALS AND METHODS

Experimental Animal:

The albino rat, *Rattus norvegicus* (Berkenhout) has been selected for the present study. The colony of the Wistar albino rats inbred at the animal house of Zoology Department was used throughout the study. The experimental albino rats were acclimated for one month prior to experiment. Adult male and female rats of almost equal size and weight ranging from 100-150g were kept in the polypropylene cages, measuring 45 x 27 x 15 cms at temperature $25^{o} \pm 0.5^{o}$ C, relative humidity 60 $\pm$ 5% and photoperiod 12 hours per 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 were 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 Lever Ltd, India and water was provided ad libitum.

Experimental Gases and Antioxidant:

Sulphur dioxide (SO_2) and Nitrogen dioxide (NO_2) gases were selected for the present study. Dietary antioxidant (tomato) was used as an antioxidant.

Experimental Protocol:

The albino rats were grouped in 3 sets-A, B, C. Control set (A) exposed to ambient air for one hour. Experimental set (B) exposed to combined SO_2 and NO_2 gas (10ppm+20ppm) for one hour per day. Experimental set (C) exposed to combined SO_2 and NO_2 gas (10ppm+20ppm) one hour per day with pre-exposure supplementation of dietary antioxidants (1g/100 g.b.wt.) for 4 weeks and 8 weeks.

Collection of Blood Samples:

Blood samples were taken directly from the ventricles of the dissected rats with the help of sterilized disposable syringe fitted with 22 SWG hypodermic needle and were taken in double oxalate vials (vide supra) for various haematological investigations. However, blood samples were analyzed individually of each rat.

Hematological parameters:

The RBCs were counted with the help of Improved Standard Neubauer Haemocytometer (Dacie and Lewis, 1975); haemoglobin concentration was determined with the help of Sahli's method described by Wintrobe *et al*. (1981) and packed cell volume was determined by Wintrobe *et al*. (1981).

RESULTS AND DISCUSSION

In the present study, the total RBC count, haemoglobin concentration and packed cell volume (PCV) decrease significantly after combined exposure to sulphur dioxide and nitrogen dioxide gas in albino rats. The decrease in the total RBC count, haemoglobin concentration may be correlated with the oxidative damage in red blood cells after inhalation of combined gases. These toxic gases make contact with internal environment of the body through respiratory tract and enter into the blood stream where come in contact with red blood cells and their contents and interfere the normal metabolism of erythrocytes. Sulphur dioxide and nitrogen dioxide binds to iron of haemoglobin and leads to the formation of sulphaemoglobin and methaemoglobin. Both sulphaemoglobin and methaemoglobin lose their ability to transport oxygen resulting tissue hypoxia which causes haemolytic anaemia in albino rats.

Present findings are in accordance with the Lal, *et al.* (1993) who have reported decrease in total RBC count and haemoglobin concentration after absorption of toxic materials of smoke like CO, sulphur dioxide and NO which causes hypoxia resulting haemolytic anaemia in rats. Similarly, Saxena (1998) has reported the decrease in the total RBC count and haemoglobin concentration due to anaemic hypoxia by the toxic action of sulphur dioxide and NO gas in albino rats.

Table 1: Total RBC count ($x10^{12}$/l) in albino rat after 4 weeks and 8 weeks combined exposure to SO_2 and NO_2 gas and supplementation of dietary antioxidant

Sets (5)	Treatment	4 weeks			8 weeks		
		Range Mean±S.Em.	Significant difference from corresponding		Range Mean±S.Em.	Significant difference from corresponding	
			Set A	Set B		Set A	Set B
Control set (A)	Ambient air	7.44-8.08 7.72±0.11			7.30-7.88 7.71±0.11		
Experimental set (B)	(10ppm SO_2+20ppm NO_2)	6.76-7.98 7.20±0.20	↓**		6.68-7.25 6.96±0.11	↓****	
Experimental set (C)	(10ppm SO_2+20ppm NO_2) + (dietary carotenoids)	6.57-7.80 7.23±0.20	↓*	↑*	6.95-7.42 7.18±0.15	↓***	↑**

Table 2: Haemoglobin concentration (g/dl) in albino rat after 4 weeks and 8 weeks combined exposure to SO_2 and NO_2 gas and supplementation of dietary antioxidant

Sets (5)	Treatment	4 weeks			8 weeks		
		Range Mean±S.Em.	Significant difference from corresponding		Range Mean±S.Em.	Significant difference from corresponding	
			Set A	Set B		Set A	Set B
Control set (A)	Ambient air	13.8-16.6 15.36±0.49			15.0-15.85 15.31±0.18		
Experimental set (B)	(10ppm SO_2+20ppm NO_2)	12.5-15.0 13.26±0.44	↓***		12.5-13.7 13.22±0.20	↓****	
Experimental set (C)	(10ppm SO_2+20ppm NO_2) + (dietary carotenoids)	12.4-14.2 13.42±0.48	↓**	↑*	13.00-14.4 13.77±0.27	↓****	↑*

Table 3: Packed cell volume (per cent) in albino rat after 4 weeks and 8 weeks combined exposure to SO_2 and NO_2 gas and supplementation of dietary antioxidant

Sets (5)	Treatment	4 weeks			8 weeks		
		Range Mean±S.Em.	Significant difference from corresponding		Range Mean±S.Em.	Significant difference from corresponding	
			Set A	Set B		Set A	Set B
Control set (A)	Ambient air	38.0-43.0 40.2±0.86			38-42 40.2±0.89		
Experimental set (B)	(10ppm SO_2+20ppm NO_2)	34-39 36±0.90	↓***		34-36 35.1±0.40	↓****	
Experimental set (C)	(10ppm SO_2+20ppm NO_2) + (dietary carotenoids)	34-40 37.0±1.00	↓**	↑*	36-38 36.8±0.39	↓****	↑**

(5) – Number of albino rats in each set
ppm – parts per million
S.Em. – Standard Error of mean

* Non-significant (P>0.05)
** Significant (P<0.05)
**** Very highly significant (P<0.001)

↓Decrease
↑Increase

Guleria (2003) has also reported a reduction in the total RBC count and haemoglobin concentration after combined exposure to sulphur dioxide and nitrogen dioxide in albino rats. Similar findings are given by Etlik and Tomur (2006) who have observed an increase in sulphaemogloin due to oxidative damage after exposure to sulphur dioxide gas in albino rats. In the present study, a decrease in PCV is correlated with decrease in the total RBC count and haemogloibin concentration resulting haemolytic anemia in albino rats. The PCV reduces in anaemic condition due to decrease in total RBC count (Guyton, 1986). Similar to the present findings, the decreases in the PCV have been reported by Baskurt, *et al.* (1989) in rats after inhalation of air pollution. Lal, *et al.* (1993) have reported decrease in PCV after inhalation of wood smoke. Present findings have been supported while Gorriz *et al.* (1996) who have reported decrease in the PCV in rats after inhalation of air pollution, while Guleria (2003) have also reported the decrease in the PCV is due to decrease in the total RBC count and haemoglobin concentration in albino rat after combined exposure to sulphur dioxide and nitrogen dioxide.

In the present study, after supplementation of dietary antioxidant carotenoids, a significant increase in the total RBC count, haemoglobin concentration and packed cell volume have observed in comparison to sulphur dioxide and nitrogen dioxide exposed rats. The modulation in hematology of albino rats after supplementation of antioxidants is due to the antioxidant defence mechanism against toxic action of combined gases. The free radicals or unstable molecules produced by oxidizing gases are known to play an important role in the development of tissue injury (Avellini *et al.*, 1995). In the biological system, antioxidants enzymes as superoxide dismutase (SOD), glutathione peroxidase (GSH) and catalase serve primary line of defense in destroying free radicals (Madhavi *et al.*, 1996). Antioxidant dietary carotenoids prevent the damaging effects of oxidants on biological system by reacting directly with reactive oxygen species (ROS) to yield non-radical products. The deleterious effects of the free radicals kept under check by a delicate balance between the rate of their production and elimination by the different antioxidant systems. Any shift in the critical balance could result in increasing peroxidation stress and may lead to cellular damage. Antioxidants could prove useful as an adjuvant treatment for blood disorders, particularly in subjects exposed to additional oxidative stress associated with oxidant pollutants (Grievink *et al.*, 1998). Some situations may lead to erythrocytes damage and haemolysis by oxidative stress. These situations can be prevented by administration of antioxidants (Toker *et al.* 1990 and Regnault *et al.* 1993). The dietary supplementation plays a modulating role on the acute effects of air pollutants by healing up the oxidative stress (Romieu *et al.*, 2008).

The present study suggests role of dietary antioxidant in combating pollution induced oxidative stress to minimize hematotoxicity.

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