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ORIGINAL ARTICLE

Assessment of Modulatory Effect of Antioxidants on Cell Shape and Size in Albino Rat after Combined Exposure of SO_2 and NO_2

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ABSTRACT

The earth is the only planet known in the entire universe capable of supporting life. In the long and tortous evolution of the human race on this planet, a stage has been reached when, through the rapid acceleration of science and technology, man has acquired the power to transform his environment. Among these, oxides of sulphur and nitrogen are most serious gaseous pollutants of urban and industrial atmosphere.Bood is an important organs to transport various components to all parts of body. The present study is designed to assess the effect of major air pollutants on blood cell morphology in albino rats and its modulation by antioxidant.
Key word: *Modulatory Effect, Antioxidants, Albino Rat, SO_2 and NO_2*

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INTRODUCTION

The need for development through socio-economic activities, especially, with the growing population and desire for higher standard of living with industrial production is obvious. Development is associated with some amount of environmental degradation. As man began to develop a settled life in small communities, the activities of clearing tree, building shelters, cultivating crops, preparing and cooking food must have altered the natural environment.Utilization of coal as fuel and the incomplete combustion of it give out sulphur and nitrogen gases in the atmosphere. The manufacture of explosives, anaerobic bacterial breakdown of nitrogenous compounds, power plants, industrial installations and nitrogenous fertilizers are the important sources of nitrogen oxides, while in metallurgical operations such as zinc, copper and lead, sulphur dioxide is evolved. Sulphuric acid plants, paper manufacturing plants and open burning of refuse and municipal incinerators also contribute sulphur dioxide in the urban atmosphere. 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.Thus, considering the fact, the present study is designed to assess the combined effects of sulphur dioxide and nitrogen dioxide gas on blood cell morphology of albino rat, *Rattus norvegicus* (Berkenhout) and protective role of antioxidant in order to minimize the combined effect of gaseous pollutants as sulphur dioxide and nitrogen dioxide. The albino rat is selected as experimental animal because of its easy adaptability and having physiological similarity to the human beings.

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 ± 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 Antioxidants:
Sulphur dioxide (SO_2) and Nitrogen dioxide (NO_2) gases were selected for the present study.Cecon drops (ascorbic acid 100mg/ml) from Pharmacia H Care Company, Bangalore was used as an antioxidant. Evion drops (tocopherol acetate 50mg/ml) from Merk Company, Aurangabad 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 combination of vitamin E (5 mg/100g.b.wt.) and vitamin C (5 mg/100g.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.

RBC Shape and Size:
The shape of the red blood cells was observed in the air dried methanol fixed blood smears.The size of the red blood cells was measured in the air dried methanol fixed blood smear by meopta015X measuring eye piece with micrometric screw adjustment. The size of cells was expressed in microns (μ).

RESULTS AND DISCUSSION

Control Set A:
The photomicrograph of blood film of control rats shows non-nucleated, circular and biconcave disc-shaped red blood cells. The red blood cells form a monolayer a paler central area occupies the middle third of the cell (Plate-I).The RBC size of control set after 4 weeks exposure to ambient air ranges from 7.32 to 8.02 μ, with an average of 7.78 μ (Table 1).

Experimental Set B (Combined Exposure to SO_2 and NO_2):
Spherocytes as small round deeply stained cells in which normal area of central paler is lost. These cells are less disc like, more spheroidal and have decreased diameter (Plate-II). Stomatocytes with linear unstained area across the centre and elliptocytes with long central axis along with normal red blood cells are also observed in the photomicrograph of blood film of albino rats after 4 weeks combined exposure to (10ppm+20ppm) sulphur dioxide and nitrogen dioxide gas (Plate-II). The RBC size after 4 weeks combined

exposure to (10ppm+20ppm) sulphur dioxide and nitrogen dioxide gas ranges from 5.90 to 8.12 μ, with an average of 7.20 μ.The decrease in the mean value of the RBC size is significant after 4 weeks combined exposure to (10ppm+20ppm) sulphur dioxide and nitrogen dioxide gas in comparison to control rats (Table 1).

Experimental Set C (Combined Exposure to SO_2 and NO_2 Along with Antioxidant Vitamin E and C):

Spherocytes and elliptocytes are disappeared, while stomatocytes are seen after 4weeks combined exposure to sulphur dioxide and nitrogen dioxide gas along with pre-exposure supplementation of antioxidant vitamins E and C in the photomicrograph of blood film of albino rats (Plate-III).The RBC size after 4 weeks combined exposure to (10ppm+20ppm) sulphur dioxide and nitrogen dioxide gas along with pre-exposure supplementation of combined antioxidant vitamins E and C ranges from 7.16 to 7.72 μ, with an average of 7.40 μ.The decrease in the mean value of the RBC size is significant after 4 weeks combined exposure to (10ppm+20ppm) sulphur dioxide and nitrogen dioxide gas along with pre-exposure supplementation of combined antioxidant vitamins E and C in comparison to control rats, while a non-significant increase in comparison to combined SO_2 and NO_2 exposed rats (Table 1).

Table 1: RBC size (μm) in albino rat after 4 weeks and 8 weeks combined exposure to SO_2 and NO_2 gas and supplementation of antioxidants

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	7.32-8.02 7.78±0.12			7.32-8.00 7.74±0.11		
Experimental Set (B)	(10ppmSO_2+20ppmNO_2)	5.90-8.12 7.20±0.20	↓**		6.74-7.38 7.05±0.10	↓****	
Experimental Set (C)	(10ppmSO_2+20ppmNO_2) + (vit. E+C)	7.16-7.72 7.40±0.09	↓**	↑*	6.98-7.64 7.34±0.11	↓**	↑**

(5)– Number of albino rats in each set * Non-significant (P>0.05) ↓Decrease
ppm– parts per million ** Significant (P<0.05) ↑Increase
S.Em.– Standard Error of mean **** Very highly significant (P<0.001)

In the present study, significant decrease in RBC size is the result of inadequate supply of oxygen after inhalation of combined gases. An increase in sulphaemoglobin and methaemoglobin is an indication of oxidative damage of RBC which alters the RBC size in albino rats. The RBC size decreases due to increase in the gas diffusion surface area so that oxygen transport is maintained despite the lower haemoglobin concentration (Wintrobe, *et al.*, 1981). Present findings are in accordance with the findings of Agarwal and Guleria (2006) who have reported the decrease in RBC size due to inadequate supply of oxygen after combined exposure to sulphur dioxide and nitrogen dioxide in albino rats. Similar to the present findings Yarmaguchi, *et al.* (1987) have stated that decrease in RBC size is beneficial to oxygen transport because gas diffusion surface area increases. Baskurt, *et al.* (1990[a]) in rats after sulphur dioxide and Baskurt, *et al.* (1990[b]) in male military students after exposure to air pollutants, have also reported that increased sulphaemoglobin might be considered as an indicator of oxidative damage of erythrocytes and as a result, size of RBC can be affected.

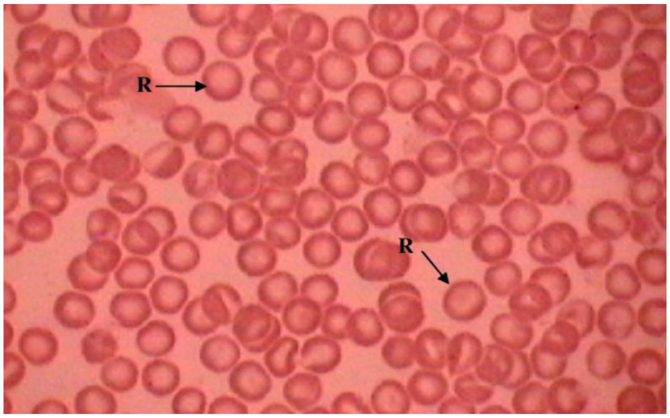

Plate I: Photomicrograph of blood film of control albino rats (1,000X)

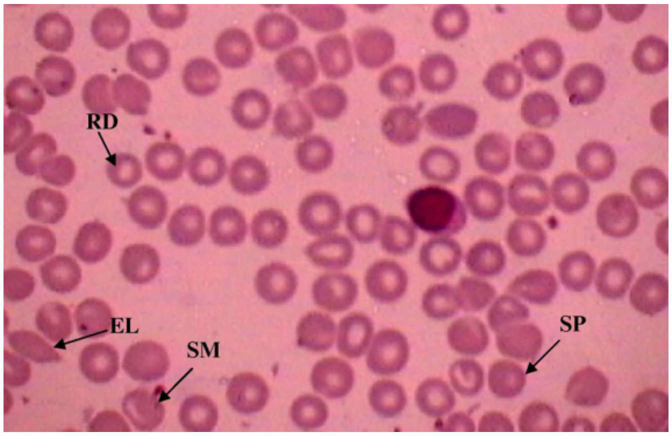

Plate II: Photomicrograph of blood film of albino rats after 4 weeks to combined exposure to (10ppm+20ppm) sulphur dioxide and nitrogen dioxide (1,000X)

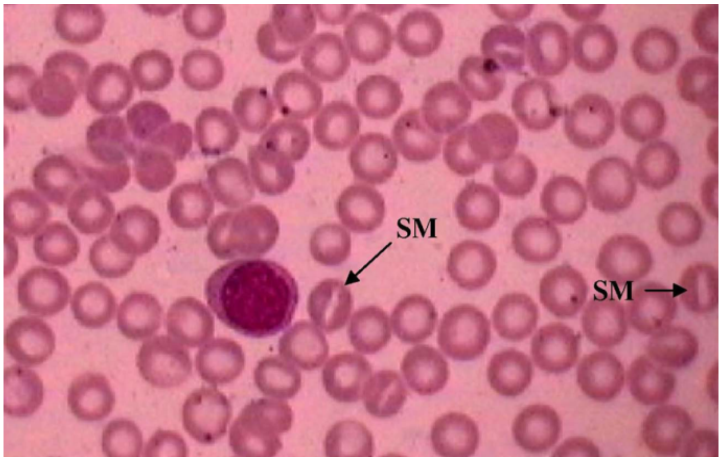

Plate III: Photomicrograph of blood film of albino rats after 4 weeks to combined exposure to (10ppm+20ppm) sulphur dioxide and nitrogen dioxide along with the pre-exposure supplementation of combined antioxidant vitamins E+C (1,000X)

R= Red blood cell, **RD=** RBC of decrease size, **SM=** Stomatocyte, **SP=** Spherocyte, **EL=** Elliptocyte

Further, present findings are supported by Nigam (2007) who has reported the decrease in RBC size in albino rats after sulphur dioxide exposure. In the present study, the appearance of stomatocytes, spherocytes, elliptocytes and dacrocytes is an indication of red blood cell membrane injury by the toxic action of combined gases. Such injury takes the form of loss of area of cell membrane and may be followed by cell haemolysis (Penington, 1978). The deteriorated cells are recognized on the blood film as stomatocytes, spherocytes, elliptocytes and dacrocytes.The stomatocytes are cells as a result of membrane abnormality, probably the expansion of the inner leaflet of the lipid bilayer of erythrocyte membrane due to the toxic action of combined gases. The membrane abnormality leads to change in passive cation and permeability and results in a hemolytic anaemia. The spherocytes have lost membrane without equivalent loss of cytosol as a result of abnormality in the red cell cytoskeleton and membrane due to oxidant gases. The progressive loss of erythrocyte membrane leads to formation of spherocytes. These changes make cell more osmotic fragile and result in haemolytic states (Boyd, 1976; Britton, *et al.*, 1989). Similar to the present findings, Agarwal and Guleria (2006) have reported spherocytesalongwithstomatocytes due to progressive loss of red cell membrane after combined exposure to sulphur dioxide and nitrogen dioxide in albino rats, while Nigam (2007) have reported stomatocytes and spherocytes after sulphur dioxide exposure in albino rats is the result of oxidative damage of membrane which leads to haemolyticanaemia.

In the support of present findings, Baskurt (1988), Baskurt, *et al.* (1989) and Baskurt, *et al.* (1990[b]) have reported the oxidative damage in erythrocyte which affects the structure of red blood cells after toxic gases. Etlik, *et al.* (1995) also reported the erythrocyte membrane may deteriorated by toxic action of sulphur dioxide gas as a result of oxidative damage in albino rats.In the present study, the appearance of elliptocytes are associated with red cell cytoskeleton abnormality which causes microcytic and haemolytic states in albino rats (*vide supra*). In the support of present findings, Britton, *et al.* (1989) have stated that elliptocytosis is a result of abnormal cytoskeleton of erythrocytes due to toxic effect of oxidant gases. The appearance of tear drop shaped dacrocytes have been reported alongwithspherocytes, stomatocytes and elliptocytes after 8weeks combined exposure in albino rats, are also associated with haemolyticanaemia which is likely to be consequent on the action of splenic macrophage on abnormal erythrocytes (Britton, *et al.*, 1989). In the present study, spherocytosis is more pronounced after prolonged exposure to sulphur dioxide and nitrogen dioxide is correlated with increase in osmotic fragility resulting in greater degree of spherocytosis in albino rats. Osmotic fragility is an indication of the shape of cells. The more fragile the cell the greater is their degree of spherocytosis (Chaudhuri, 1993).

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