

COMBINED EFFECTS OF SO₂ AND NO₂ GAS ON BLOOD FEATURES OF ALBINO RAT

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*Abstract: The objective of present investigation was to evaluate combined effects of SO₂ and NO₂ gas on blood features of albino rat. The haematological parameters {total erythrocyte count, haemoglobin concentration, packed cell volume (PCV), red cell indices-mean corpuscular volume, mean corpuscular haemoglobin concentration, total leucocyte count and differential leucocyte count (DLC)} of albino rat, *Rattus norvegicus* (Berkenhout) were evaluated after combined exposure to 40 ppm NO₂ + 40ppm SO₂ for 15 and 30 days for one hour per day. A highly significant decrease in the total erythrocyte count (TEC), haemoglobin concentration (Hb. Conc.) and packed cell volume (PCV) and total leucocyte count (TLC), red cell indices, neutrophil count, monocyte count have been observed. A non-significant decrease in the eosinophil count and basophil count and very highly significant increase in the lymphocyte count have observed after combined exposure to these gases.*

Key words: SO₂, NO₂, Blood features, Albino rat

INTRODUCTION

Pollutants may be either solid, liquid or gaseous form and affect air quality and cause hazardous to life. Air pollution is one of dangerous problem of present society. The common air pollutants are oxides of sulphur and nitrogen, carbon dioxide, carbon monoxide, ammonia, hydrocarbons and particulates. Among these, oxides of sulphur and nitrogen are most serious gaseous pollutants of urban and industrial atmospheres. High concentration of nitrogen dioxide and sulphur dioxide gas is able to transport deep in the lung from where these gases enter the blood stream. Blood is an index of health of organism. Any change in the blood tissue can alter the body metabolism. Thus, considering the fact, present study is designed to access the combined effect of nitrogen dioxide and sulphur dioxide gas on haematological parameters of albino rat, *Rattus norvegicus* (Berkenhout). The albino rats were exposed to 40 ppm + 40 ppm of nitrogen dioxide and sulphur dioxide gas for 15 days and 30 days for one hour.

MATERIALS AND METHODS

Adult albino rat of both the sexes of almost equal size and weight ranging from 100-150 gm, were kept in polypropylene cages at 25 ± 0.5 °C temperature and relative humidity 60 ± 5% and acclimated for one month prior to experiment. The rats were fed on gold mohar rat feed, manufactured by Hindustan Lever Ltd., India, at fixed time intervals and water was provided *ad libitum*. The nitrogen dioxide and sulphur dioxide gas generator and fumigation chamber (Model AP-07, SFC-120) manufactured by Standard Appliances, Varanansi, were used for exposure of albino rat to nitrogen dioxide and sulphur dioxide. The NO₂ gas was generated in NO₂ generator by the controlled action of conc. nitric acid on copper turnings [1,2]. SO₂ gas was generated in SO₂ generator by the controlled action of 5% sulfuric acid and sodium sulphite [3].

The experimental albino rats were grouped into 2 sets randomly, one control set 'A' and experimental

set 'B' of ten rats each. The rats of control set 'A' were exposed to ambient air for 15 days and 30 days for one hour per day, while experimental sets 'B' was exposed to 40 ppm + 40 ppm NO₂ and SO₂ combined gases respectively for one hour per day for 15 days and 30 days in the fumigation chamber. At the end of exposure period, rats were dissected and blood sample was obtained directly from the ventricle of heart in oxalated vials for the estimation of haematological parameters viz. TEC, Hb. Conc., PCV, MCH, MCV, MCHC, TLC, by using automatic analyzer. The differential leucocytes were counted on the air dried and methanol fixed blood smear stained by Leishman stain and results were expressed in per cent (%). The readings so obtained were subjected to the formulae for different statistical calculations [4].

RESULTS AND DISCUSSION

The results obtained for the haematological parameters after combined exposure to NO₂ and SO₂ are shown in Tables 1 and 2. Study shows total erythrocyte count and haemoglobin concentration decrease significantly after 15 days and 30 days to

40 ppm + 40ppm combined exposure to nitrogen dioxide and sulphur dioxide gas. The significant reduction in the total RBC count and haemoglobin concentration is due to toxic effect of combined gases on the haemopoietic tissue. Combined gases make contact with the internal environment of body through respiratory tract and interfere the metabolism of red blood cell and its haemoglobin contents which results hemolytic anaemia in albino rats. Inadequate supply of oxygen to red blood cell due to inhalation of toxic gases causes oxidative damage in red blood cells and consequently resulting in the formation of sulphaemoglobin and methaemoglobin. This represents an irreversible change in haemoglobin pigments lacking oxygen carrying capacity of the blood and causes anaemic hypoxia. In the support of present findings, Case et al. [5] have reported the metabolic alterations in erythrocytes leads to formation of the methaemoglobin in rats after exposure to oxidant air pollutants, while Medeiros et al. [6] have reported an increase in sulphaemoglobin and methaemoglobin ratio due to the influence of air pollutants. Methaemoglobin is incapable of transporting oxygen and thus causes cyanosis in mice and rats. An increased rate of oxidation of

Table-1: Haematological parameters in albino rats after 15 and 30 days combined exposure to NO₂ and SO₂ gas. * Non-significant (P>0.05), ** Significant (P<0.02), *** Highly significant (P<0.01), **** Very highly significant (P<0.001)

Parameters	Exposure days	Control set (5)	Experimental set (5)
			40ppm NO ₂ + 40ppm SO ₂ gas
		Range Mean ± S. Em.	Range Mean ± S. Em.
TEC (x10 ¹² /l)	15	8.07-9.88 9.07 ± 0.29	7.08-8.56 7.78 ± 0.23****
	30	8.40-9.42 8.96 ± 0.16	6.77-8.00 7.51 ± 0.25****
Hb.Conc. (gm/dl)	15	16.8-18.0 17.32 ± 0.19	12.6-14.8 13.64 ± 0.44***
	30	15.6-17.2 16.44 ± 0.33	11.6-12.6 12.16 ± 0.1****
PCV (%)	15	46.0-53.0 49.60 ± 1.12	38.0-44.0 40.8 ± 0.96****
	30	46.0-50.0 48.0 ± 0.70	34.0-40.0 37.80 ± 1.11****
MCV (fl)	15	53.19-57.00 54.76 ± 0.66	51.40-53.67 52.42 ± 0.46**
	30	52.45-54.76 53.57 ± 0.40	46.40-54.0 50.25 ± 0.99***
MCH (pg)	15	18.21-20.81 19.15 ± 0.45	16.9-18.41 17.51 ± 0.25****
	30	18.25-19.00 18.47 ± 0.14	15.54-17.19 16.23 ± 0.38**
MCHC (gm/dl)	15	34.23-36.50 34.94 ± 0.41	33.39-34.50 33.39 ± 0.24*
	30	34.23-34.79 34.48 ± 0.11	31.30-32.32 31.78 ± 0.19**

Table 2: Haematological parameters in albino rats after 15 and 30 days combined exposure to NO₂ and SO₂ gas. * Non-significant (P>0.05), ** Significant (P<0.02), *** Highly significant (P<0.01), **** Very highly significant (P<0.001)

Parameters	Exposure days	Control set (5)	Experimental set (5)
			40ppm NO ₂ + 40ppm SO ₂ gas
		Range Mean ± S.Em.	Range Mean ± S.Em.
TLC (x10 ⁹ /l)	15	7.95-8.95 8.43 ± 0.20	6.25-7.90 7.10 ± 0.31***
	30	7.80-8.90 8.46 ± 0.18	4.15-6.05 5.23 ± 0.31***
Lymphocyte (%)	15	49-51 49 ± 0.44	53-55 54.0 ± 0.44 **
	30	49-51 50.8 ± 0.37	52-61 56.8 ± 1.46 ***
Monocyte (%)	15	4-7 5.6 ± 0.50	2-6 3.8 ± 0.66*
	30	4-8 5.8 ± 0.66	2-5 3.4 ± 0.50**
Neutrophil (%)	15	40-43 41.6 ± 0.50	40-41 40.2 ± 0.21**
	30	39-44 41.6 ± 0.92	36-41 38.2 ± 0.84**
Eosinophil (%)	15	1-3 1.8 ± 0.37	0-3 1.4 ± 0.50*
	30	1-3 1.8 ± 0.37	1-3 1.2 ± 0.48*
Basophil (%)	15	0-2 1 ± 0.31	0-1 0.6 ± 0.24*
	30	0-2 1 ± 0.31	0-1 0.4 ± 0.24*

haemoglobin to methaemoglobin in man after exposure to nitrogen dioxide [7-9]. Similarly, the decrease in the total RBC count and haemoglobin contents have been given by Baskurt et al. [10] in human, Lal et al. [11] in rats, after exposure in air pollution and smoke like CO, SO₂ and NO etc. respectively.

In the present study, the packed cell volume decreases very highly significantly after 15 days and 30 days exposure to sulphur dioxide and nitrogen dioxide. A fall in the packed cell volume is correlated with haemolytic anaemia in albino rats due to decrease in the total RBC count and haemoglobin concentration after combined exposure to toxic gases. Packed cell volume reduces in anaemia [12]. Lesser the RBC count, the lesser is the packed cell volume [13]. Almost similar observation have been made by Baskurt et al. [10] after exposure to air pollution, Lal et al. [11] after smoke, Gorriz et al. [14] after exposure to air pollution in rat.

The study reveals red cell indices the mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration decrease after combined exposure to nitrogen dioxide and sulphur dioxide gas. The values of red cell indices mainly depend on the values of total RBC count, haemoglobin concentration and packed cell volume. The reduction in the values of MCV, MCH and MCHC is due to decrease in the values of total RBC count, haemoglobin concentration and packed cell volume. The decrease in the values of red cell indices indicate the hypochromic microcytic anaemic condition [15]. The decrease in mean corpuscular volume have also been reported by Baskurt [16] after air pollution exposure, while decrease in mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration have been reported by Baskurt and Balkanci [17] due to effect of air pollution .

In the present study, a very highly significant decrease in the total WBC count after 15 days and 30 days combined exposure to nitrogen dioxide and sulphur dioxide is observed. A decrease in the total WBC count may be due to massive migration of leucocytes to the site of tissue injury leads to the reduction of total WBC count in peripheral blood resulting leucopenia with anaemia. Similar observations regarding decrease in total WBC count in rats have been made by Agarwal [18] who stated that a fall in white blood cell count is an indication of toxic effects

of combined gas sulphur dioxide and nitric oxide on the haemopoietic system causes leucopenia with anaemia in albino rats.

Present findings are further supported by Alarie et al. [19] in cynomolgus monkey, Lal et al. [11] in rats. They also reported decrease in the total WBC count after SO₂ and wood smoke exposure respectively. In the present investigation, the differential leucocyte count show a very highly significant increase in the percentage of lymphocyte count, while significant decrease in the percentage of neutrophil count and monocyte count after 15 and 30 days combined exposure to nitrogen dioxide and sulphur dioxide gases. However, percentage of eosinophil count and basophil count shows non-significant decrease in the albino rats. The increase in lymphocyte count may be due to the irritant effect of combined gases on the respiratory tract which cause lymphocytosis. Present findings are in accordance with the findings of Nancy et al. [20] who reported an increase in lymphocytes in human due to irritant effect of cigarette smoking. Another possible explanation for elevation of lymphocyte percentage may be the disturbance in adrenal gland under the influence of stress factor like toxic gases like nitrogen dioxide and sulphur dioxide. Similar views are given in the study of human [20] and rats [21,22]. An increase in percentage of lymphocytes have also reported by Ehrman et al. [7] in mice and Ramage et al. [23] in human after exposure to nitrogen dioxide and wood smoke respectively.

A significant decrease in the percentage of neutrophils and monocytes may be due to the immigrant macrophages at the sites of inflammation appear to come from blood , so that the blood neutrophil and monocyte percentage decreases [24]. Similar to present findings, the decrease in the percentage of monocyte have been reported by Nancy et al. [20] in human, Srivastava et al. [25] in rats after cigarette smoking and fly ash exposure respectively .Similar findings are given by Agarwal and Nigam [22] who have reported leucocytosis, neutropenia with relative monocytopenia after exposure sulphur dioxide and nitrogen dioxide in albino rats.

Toxicity of combined exposure of sulphur dioxide and nitrogen dioxide is exposure time dependent so alterations in haematological parameters are more pronounced after 30 days combined exposure to sulphur dioxide and nitrogen dioxide.

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