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Research Paper

Combined effect of vitamin C and E against the toxicity of Nitrogen dioxide gas on Total Erythrocyte Counts in Albino rats

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Abstract: In the present investigation, total erythrocyte counts have been observed after nitrogen dioxide gas (50ppm) exposure in both the sexes of albino rats for one hour per day for 15 and 30 days. Nitrogen dioxide gas toxicity induces hypoxic polycythemia due to oxidative damage, which increases the number of red blood cells in both the sexes of albino rats. Male and female rats of equal size and weight (150-200g) were kept in standard laboratory conditions and grouped into three sets (I, II, & III) containing twelve rats each. Control set (I) was unexposed, Experimental set (II) was exposed to nitrogen dioxide gas (50ppm) and Experimental set (III) was exposed to nitrogen dioxide gas (50ppm) along with supplementation of vitamin C (5mg/rat) and E (2.5 mg/rat) in combination for one hour per day for 15 and 30 days. Total erythrocyte counts were declined after supplementation of vitamin C and E in combination in comparison to nitrogen dioxide gas exposed rats of both the sexes due to antioxidants defense mechanism.

INTRODUCTION

Air pollutants are emitted directly from identifiable sources and by air borne compounds include the substance in sufficient concentration to produce a measurable effect on man or other animal vegetation or material. Nitrogen dioxide gas is a main component of air pollution. Its inhalation is responsible for severe breathlessness and inflammation. High concentration of this gas transports deep in lungs from where it enters directly to the blood stream affecting total cell count, in which total erythrocytes counts are the major cell count in blood, produced by megakaryocytes of bone marrow. The toxic effect of air pollutants can be mitigated by the supplementation of antioxidants because they scavenge the free radicals species generated by pollutants. So keeping this in view present study is carried out on total erythrocyte counts in both the sexes of albino rats exposed to nitrogen dioxide gas and supplementation of vitamin C and E in combination.

MATERIALS AND METHODS

Thirty six (36) adult male and female wistar albino rats (150±25g) were taken for the present study and they were kept in polypropylene cages in standard conditions of temperature 25±0.5^{o}C, relative humidity 60±5% and photoperiod of 12 hours /day, Rats were fed on pallet diet (Golden feed, New Delhi, India) and water *ad libitum*. Experimental animals were acclimated for two weeks prior to the experiment. Evion drops as vitamin C (5 mg/rat) and vitamin E (2.5 mg/rat) from Merck Company, Aurangabad were used as antioxidants. The nitrogen dioxide gas was prepared by Saltzman method (1954) and modified by Levaggi *et al*. (1972). All the experiments were carried out as per guidelines of Institutional Ethical committee.

Experimental Protocol

Control set (I) and Experimental sets (II and III) containing six male and six female rats in each set.

Control set (I_m&I_f) not exposed to nitrogen dioxide gas.

Experimental set (II_m&II_f) Exposed to nitrogen dioxide gas (50 ppm) for one hour per day for 15 and 30 days.

Experimental set (III_m&III_f,) Exposed to nitrogen dioxide gas along with supplementation of antioxidant vitamin C (5mg/rat) and vitamin E (2.5 mg/rat) in combination for one hour per day for 15 and 30 days.

Exposure to nitrogen dioxide gas:- Experimental male and female albino rats were exposed to nitrogen dioxide gas in a fumigation chamber (model AP-07, SPC-120) manufactured by standard Appliances Varanasi. Rats were subjected to the whole body exposure for one hour per day For 15 and 30 days.

Sample collection

Six rats (3 male and 3 female) of each set (I, II and III) were sacrificed after 15 days and remaining after thirty days. Blood samples were collected directly from the ventricles of the heart of the dissected rats with the help of sterilized disposable syringes and were taken into double oxalate vials.

Total erythrocytes counts were done with the help of improved standard Neubauer Haemocytometer (Dacie and Lewis, 1968).

The data obtained from the observations were subjected to a one way ANOVA test KPky plot (ver 3.0)

RESULTS AND DISCUSSION

The values of total erythrocytes count in control set (I) and Experimental set (II) and set (III) for 15 and 30 days are given in table (I).

In the present study, a significant increase in total erythrocyte count is correlated with oxidative damage of erythrocytes after inhalation of nitrogen dioxide gas. An inadequate supply of oxygen to the tissues act as a stimulus for marrow erythrocyte production is the result of hypoxic polycythemia in both the sexes of albino rat. Erythrocytosis usually results from massive release of erythropoietin by tissue deprived of oxygen.

Present findings gain support with the findings of Guidotti (1980) who have reported that erythropoiesis produces hypoxic polycythemia after exposure to nitrogen dioxide gas in rats. Wilcox *et al*. (1993) have observed erythropoiesis inducing polycythemia in humans after nitric oxide exposure. Similar observations are also made by Kawata *et al*. (1998) and Walker *et al*. (2000) who have noted nitric oxide inhalation causes chronic hypoxia stimulating erythropoiesis and resulting polycythemia in rats. Sharon *et al*. (2000) have also reported an increase in erythrocyte due to nitrogen dioxide gas toxicity.

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Table: Total Erythrocyte Count ($x10^6$/µl) in both the sexes of albino rat after 15 and 30 days NO_2 exposure and supplementation with antioxidants

Exposure	Sets	Exposure	Male			Female		
			Range Mean± S.Em.	Significance difference from corresponding		Range Mean± S.Em.	Significance difference from corresponding	
				Control Set– I_M	Experimental Set– II_M		Control Set– I_F	Experimental Set– II_F
15 days	Control sets– I_M&I_F (5)	Ambient air	6.24–7.38			5.85–6.88		
			6.99±0.20			6.30±0.21		
	Experimental sets							
	Set– II_M&II_F (5)	50ppmNO$_2$	7.47–8.87	P<0.05 ↑**		6.00–6.79	P<0.05 ↑**	
			8.21±0.26			6.56±0.14		
	Set– III_M&III_F (5)	50ppmNO$_2$+vit. C+E	6.62–7.64	P>0.05 ↓*	P<0.01 ↓***	5.42–6.72	P>0.05 ↓*	P<0.05 ↓**
			7.04±0.12			5.96±0.21		
30 days	Control sets– I_M&I_F (5)	Ambient air	6.34–7.86			5.82–6.34		
			7.33±0.27			6.05±0.08		
	Experimental sets							
	Set– II_M&II_F (5)	50ppmNO$_2$	7.33–8.87	P<0.05 ↑***		6.67–7.00	P<0.001 ↑**	
			8.19±0.28			6.82±0.18		
	Set– III_M&III_F (5)	50ppmNO$_2$+vit. C+E	6.98–7.62	P>0.05 ↓*	P<0.05 ↓**	5.90–7.80	P>0.05 ↑*	P>0.05 ↓*
			7.30±0.13			6.80±0.37		

ppm = parts per million
(5) = Number of albino rats
M = Male, F = Female
S.Em. = Standard Error of mean

*Non–significant (P>0.05)
**Significant (P<0.05)
***Highly–significant (P<0.01)
**** Very highly significant (P<0.001)

↓Decrease
↑Increase

In the present study, the reduction in toxic effect of nitrogen dioxide gas on the total erythrocytes count in both the sexes of albino rat after supplementation of vitamin (C+E) is due to antioxidant defense mechanism against nitrogen dioxide gas induced oxidative stress and inflammation. Antioxidant suppresses the oxygen free radicals production and a beneficial protectants against oxidative damage (Anonymous, 1996). Present findings are also gain support by Clarkson and Thompson (2000) who found that antioxidants (vitamin C+E) in combination can reduce indicators of oxidative stress as a result of exercise at the very primary stage. Similar to the present findings, Schwela (2000) have cited the evidence that vitamin C and E both decreases the effect of air pollution, helps in wound healing, maintain cellular oxygen turnover and protect against inflammatory disorders by demonizing free radicals that form in fatty tissues.

Conclusion: In the present investigation the toxicity of nitrogen dioxide gas induces disorders in total erythrocyte counts in both the sexes of albino rats, but parallel with that supplementation of antioxidants vitamin C and E shows ameliorative impact in reducing the pollutants effect.

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