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REVIEW ARTICLE

ALTERATIONS IN TOTAL LEUKOCYTE COUNTS AFTER INHALATION OF NITROGEN DIOXIDE GAS IN ALBINO RATS AND MODULATION BY VITAMIN C AND E SUPPLEMENTATION

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ABSTRACT

In the present study, albino rats of both the sexes were exposed to nitrogen dioxide gas (50ppm) for one hour per day for 15 and 30 days. Albino rats of both the sexes of equal size and weight (150-200g) were kept in standard laboratory conditions and grouped in three sets (A, B and C) containing twelve rats each. Control set (A) was unexposed, experimental set (B) was exposed to nitrogen dioxide gas (50ppm) and experimental set (C) was exposed to nitrogen dioxide gas (50ppm) along with supplementation of vitamin C (5mg/rat)and E (2.5mg/rat) for one hour per day for 15 and 30 days. Total leukocyte counts increases significantly after nitrogen dioxide gas inhalation in both the sexes of albind rat of albino rat, due to irritant effect of toxic gas and airway inflammation which induces Leukocytosis. Supplementation of antioxidants vitamin C and E plays a protective role in attenuating the toxic effect of NO_2 gas in both the sexes of albino rat due to antioxidant defense mechanism to a greater extent.

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INTRODUCTION

There are abundant pollutants which are exist in environment but day by day they are interlinking with a very high speed, among these pollutants nitrogen dioxide gas is a chief air pollutant often released into the environment as a byproduct of fuel combustion but rarely released by spontaneous combustion, known sources of nitrogen gas includes automobiles exhaust and power stations and spread its toxic effects in form of generating oxygen reactive species (free radicals). They are introducing to human being by environmental oxygen inhaling by nasal passage from where these pollutants are mixed with blood and get in touch with body's internal environment by disturbing whole body's blood cell functioning in which total leukocyte counts are the main cell counts, are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. All white blood cells are produced and derived from multipotent cells in the bone marrow known as hematopoietic stem cells. Leukocytes are found throughout the body, including the blood and lymphatic system (Maton *et al*., 1997). But parallel with that there is a way to protect from pollutants by using antioxidants vitamin C and E in mitigating the toxic effects of pollutants.

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So considering this, present study is carried out on total leukocyte counts in albino rats exposed to nitrogen dioxide gas and administration of vitamin C and E in combination.

MATERIALS AND METHODS

Thirty six (36) adult male and female wistar albino rats (150-200g) were taken for the present study and they were kept in polypropylene cages in standard conditions of temperature 25+0.5c, 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. Vitamin C (5mg/rat) and vitamin E (2.5mg/rat) from Merck Company, Aurangabad were used as antioxidants. The nitrogen dioxide gas was prepared by *Saltzman* method (1954) and modified by *Levaggi* (1972). All the experiments were carried out as per guidelines of Institutional Ethical committee.

Experimental Protocol

Control set (A) and Experimental sets (B and C) containing six male and six female rats in each set.

Control set (A): not exposed to nitrogen dioxide gas.

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

Total Leukocyte Count ($x10^3/\mu l$) in both the sexes of albino rat after 15 and 30 days NO_2 exposure and supplementation with antioxidants Vitamin C and E

Exposure	Sets	Exposure	Male			Female		
			Range Mean±S.Em.	Significance difference from corresponding Control Set– I_M	Significance difference from corresponding Experimental Set– II_M	Range Mean±S.Em.	Significance difference from corresponding Control Set– I_F	Significance difference from corresponding Experimental Set– II_F
15 days	Control sets–I_M&I_F	Ambient air	8.8 – 9.9			6.8 – 7.7		
	(5)		9.2±0.22			7.3±0.18		
	Experimental sets							
	Set– II_M&II_F	50ppmNO_2	8.8 – 10.9	P>0.05 ↑*		6.7 – 8.4	P>0.05 ↑*	
	(5)		9.86±0.39			7.78±0.30		
	Set– III_M&III_F	50ppmNO_2+vit.	8.0 – 9.4	P>0.05 ↓*	P>0.05 ↓*	5.20–7.41	P>0.05 ↓*	P<0.05 ↓**
	(5)	C+E	8.90±0.24			6.5 ±0.40		
30 days	Control sets–I_M&I_F	Ambient air	8.0 – 9.5			6.8 – 7.6		
	(5)		8.8±0.27			7.1±0.14		
	Experimental sets							
	Set– II_M&II_F	50ppmNO_2	8.4 – 10.7	P<0.05 ↑**		6.8 –8.51	P<0.05 ↑**	
	(5)		9.54±0.44			7.6±0.33		
	Set– III_M&III_F	50ppmNO_2+vit.	7.1 – 9.7	P>0.05 ↓*	P>0.05 ↓*	5.40–7.61	P>0.05 ↓*	P<0.05 ↓**
	(5)	C+E	8.46±0.53			6.5 ±0.42		

ppm = parts per million*Non–significant (P>0.05) ↓Decrease (5) = Number of albino rats**Significant (P<0.05)↑Increase M = Male, F = Female***Highly–significant (P<0.01) S.Em. = Standard Error of mean**** Very highly significant (P<0.001)

Experimental set (C): Exposed to nitrogen dioxide gas (50 ppm) along with supplementation of antioxidant vitamin C (5mg/rat) and vitamin E (2.5mg/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 .The rats were subjected to the whole body exposure for one hour per day For 15 and 30 days.

Sample collection

Six rats (3male and 3female) of each set (A, B and C) 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 Leukocyte 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 Leukocyte Counts in control set (A) and Experimental set (B) and set (C) for 15 and 30 days are given in Table (I). In the present study, total leukocyte count increase significantly after nitrogen dioxide inhalation in both the sexes of albino rat. A significant elevation in total leukocyte count is the outcome of airway inflammation by the irritant effect of nitrogen dioxide gas in both the sexes of albino rat, which induces leucocytosis. Present findings are confirms with the findings of Solomon *et al*. (2000) who reported that nitrogen dioxide cytotoxicity causes airway inflammation which increases blood leucocytosis in rats. Wane *et al*. (2000) have also noted elevation of leucocytes caused by air pollutants in human. In add support to present findings, Agarwal and Nigam (2006) have stated that increase in WBC is due to irritant effect of sulphur dioxide exposure in albino rats. Etlik and Tomur (2006) have reported an increase in WBC by inhalation of sulphur dioxide gas which causes inflammation in rats.

In the present study, the attenuation in toxic effect of nitrogen dioxide gas on the total leukocyte count in both the sexes of albino rats after supplementation of vitamin C and E in combination is due to antioxidant defense mechanism against nitrogen dioxide which gas induced oxidative stress and inflammation. As there is evidence that vitamin C+E both work together synergistically and prevent cell destruction (Beyor, 1994, Chen and Tappel, 1995 and Lass and Shoal, 1995). In support of present findings, Senturk *et al*. (2005) have reported that vitamin C and E reduced inflammatory responses related to exhausting exercise. Similar results have also given by Achuba (2005) who stated that toxic effect of petroleum may be reduced by supplementation of vitamin C and E by playing a protective role.

Conclusion

Result of present study shows that vitamin C and E exhibitated a beneficial impact in reducing the toxic effect of nitrogen dioxide gas in albino rats.

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