Effect of Nitrogen dioxide on platelet counts of Albino rats: Related to sex and role of Antioxidants

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Abstract - The present investigation was carried out to determine the effect of nitrogen dioxide and role of vitamin C (5mg/rat) and vitamin E (2.5mg/rat) supplementation in combination on the platelet count, nitrogen dioxide in both the sexes of albino rats. Experimental rats were kept in standard laboratory conditions and grouped in three sets containing three male and three female rats. Each-control (set A) without exposure, experimental set (B) was exposed to nitrogen dioxide gas(50 ppm) alone, while experimental set (C) was exposed to nitrogen dioxide gas along with supplementation of vitamin E and C in combination for two and four weeks for one hour per day. The results of the present study imply that the platelet count elevated after nitrogen dioxide gas exposure in both the sexes of albino rats. While reduction in platelet count after supplementation of vitamin E and C in combination which shows the modulated effect against the toxic action of nitrogen dioxide gas exposure in both the sexes of albino rats.

Keywords: Albino rats, Nitrogen dioxide, Platelet counts, Gas exposure, Vitamins(C+E).

I. INTRODUCTION

Nitrogen dioxide is a major air pollutant exists in the atmosphere. It enters the respiratory passage from where it intermixed to the blood system and its components. By mixing with the body's internal environment, it alters the symmetry of blood parameters Blood platelet count, is a major cell count, which play a significant role in clotting mechanism without this factor clotting does not occur. Nitrogen dioxide toxicity may alter the symmetry of blood parameters while supplementation of vitamin (E+C) in combination can mitigate the toxic effect of nitrogen dioxide gas in both the sexes of albino rats.

II. MATERIALS AND METHODS

Eighteen (18) 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 *adlibitum*. Experimental animals were acclimated for two weeks prior to the experiment. Evion drops as vitamin C (5mg/rat) and vitamin E (2.5mg/rat) from Merck Company, Aurangabad were used as antioxidants. All the experiments were carried out as per guidelines of Institutional Ethical committee.

Experimental Protocol

Control set (A) and Experimental sets (Band C) containing three male and three 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 four and eight weeks.

Experimental set (C) Exposed to nitrogen dioxide gas along with supplementation of antioxidant vitamin C (5mg/rat) E(2.5mg/rat) in combination for one hour per day for four and eight weeks.

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 four and eight weeks.

III. SAMPLE COLLECTION

Six rats of control set (A), Set (B) and set (C) were sacrificed after four and remaining six after eight weeks. Blood samples were collected directly from the ventricles of the heart of the dissected rat with the help of sterilized disposable syringes fitted with hypodermic needles and were taken into double oxalate vials.

Platelet counts were done with the help of improved standard Neubauer Haemocytometer1.

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

IV. RESULTS AND DISCUSSION

The values of platelet counts in control set (A) and experimental set (B and C) for four and eight weeks are given in Table (I).

A significant decrease in platelet count in both the sexes of albino rats is due to toxic effect of nitrogen dioxide gas on the homeopathic system which causes rapid destruction of platelet count in peripheral blood, possibly associated with impairment of platelet formation by mega karyocytes resulting thrombocytopenia in both the sexes of albino rats. Release of multiple coagulation factors from the large number of platelets entrapped in fibrin mesh clot ,failure of clot retraction is an indication of decreased number of platelet in circulating blood[2]. But a significant increasehas been noticed after supplementation of antioxidants (vitamin E+C) in combination to a greater extent.

In the present study ,the reduction in toxic effect of nitrogen dioxide gas on the platelet count in male and female albino rats after supplementation of (vit.E+C) as antioxidants, is due to antioxidant defense mechanism against nitrogen dioxide gas induced oxidative stress and inflammation .

TABLE–I
PLATELET COUNT ($X10^3/\mu L$) IN BOTH THE SEXES OF ALBINO RAT AFTER 4 AND 8 WEEKS 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
4 weeks	Control sets– I_M&I_F (5)	Ambient air	856 – 977			698 –793		
			924±12.25			742±9.75		
	Experimental sets							
	Set– II_M&II_F (5)	50ppmNO_2	786 –814	P<0.001 ↓***		578 – 688	P<0.01 ↓***	
			802±4.80			635±20.63		
	Set– III_M&III_F (5)	50ppmNO_2 +vit. C+E	850 – 950	P>0.05 ↓*	P<0.01 ↑***	687 – 777	P>0.05 ↓*	P<0.05 ↑**
			893±16.51			736±18.03		
8 weeks	Control sets– I_M&I_F (5)	Ambient air	880 –945			646 – 744		
			913±6.54			700±16.77		
	Experimental sets							
	Set– II_M&II_F (5)	50ppmNO_2	672 – 728	P<0.001 ↓****		476 – 586	P<0.001 ↓****	
			697±10.69			519±19.39		
	Set– III_M&III_F (5)	50ppmNO_2 +vit. C+E	700 –850	P<0.001 ↓****	P<0.05 ↑**	526 – 650	P<0.01 ↓***	P>0.05 ↑**
			778±24.4			588±20.13		

ppm = parts per million
↓Decrease, ↑Increase
(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)

Nitrogen dioxide gas induced declination in platelet count is accompanied with inflammation in male and female albino rats .platelet disorders are characterized by increased platelet consumption produce thrombocytopenia3.similar to the present findings Kobayashi *et al*[4]. have noted decrement in platelet count production in rats after exposure to nitrogen dioxide gas. Similar views regarding decrement in platelet count have been given by Canizers *et al*[5].in male and female albino rats after exposure to nitric oxide. A reduction in platelet count aggregation has also been reported by Albert *et al*[6]. in human donors after exposure to nitric oxide also stated by Sharon *et al*[7].in rats after nitric oxide inhalation.

Present findings have also been reported by Robson *et al*[8]. that beta carotene is a powerful antioxidant, protecting cells of the body from damage caused by free radicals and enhance immunity system9. Also observed that many fruits and vegetables reduced platelet clumping[10]. Who reported that the dietary form of antioxidant play a modulating role on the acute effect of air pollutants by healing up the oxidative stress.

Antioxidant vitamin (E+C) in combination acts very quickly and decrease the net intensity of inflammation caused by free radicals, they rapidly break the chain of free radicals and have a beneficial effect against oxidative stress.

Powers and Hamilton[11], stated that vitamin (E+C) protected against stroke and prevent blood clotting there is also an evidence that vitamin (E+C) both work together synergistically and prevent cell destruction12[-14]. Similar observation also made by Jendryozko *et al*[.15] has stated that vitamin (E+C) significantly reduced platelet aggregation and blood clotting.

Present findings also get support by Schwela16 have cited the evidence that vitamin (E+C) both decreased the effect of air pollution, helps in wound healing, maintain cellular oxygen turnover and protect against inflammation disorders by demolishing free radicals.

The results of present investigation suggest that the supplementation of vitamin E and C in combination attenuated the toxic effects of nitrogen dioxide gas to a greater extent.

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