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Modulatory effect of vitamin E and C on SO_2 induced hematological alterations in albino rats

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Abstract: *The present study aimed to investigate the alterations in selected hematological parameters (total erythrocyte count, total leucocyte count, hemoglobin concentration, packed cell volume, erythrocyte sedimentation rate and red cell indices(MCV, MCH, MCHC) of wistar albino rats after short term (80 ppm, 1h/d for 28 days) exposure to SO_2 gas and protective effect of vitamin E (2.5mg/rat) and C (5mg/rat) individually and in combination. The results of the present study imply that hematological parameters altered after SO_2 gas inhalation and lead to reduction in TEC, Hb.Conc., PCV and red cell indices, while elevation in TLC and ESR. However, SO_2 induced hematological alterations modulated after supplementation of vitamin E and C individually and in combination due to antioxidant defense mechanism against toxic action of SO_2 inhalation. The findings of the present study exhibit that the supplementation of vitamin E and C in combination have more potential to mitigate SO_2 induced hematological alterations in albino rats than the supplementation of vitamin E and C individually.*

Key Words: SO_2 gas, Hematological alterations, Albino rats, Vitamin E and C.

Introduction

Sulphur dioxide is the most common air pollutant in urban atmosphere. It arises mainly from industrial processes and domestic combustion of fossil fuels. It reacts with water, oxygen and other materials in the air to form sulphur containing acids. Epidemiological studies have linked SO_2 exposure to many respiratory diseases, including lung cancer (Andersson *et al.*, 1998). It is often associated with particulate concentration and aerosol particles, are able to transport deep in the lung from where it enters the blood and can alter the body metabolism (Tewari and Shukla, 1991). Alteration of hematological parameters occurs after SO_2 gas exposure which may lead to anaemic state. Formation of sulfhemoglobin iron deficiency, dietary deficiency, increased demand and insufficient erythropoiesis are the main mechanism that have been proposed for explaining this situation. Exposure of pollutants and development of certain pathological conditions lead to oxidative stress, consequently increase production of free radicals. These free radical cells are very unstable and they attack healthy cells to replace missing electrons.

Antioxidants are capable of stabilizing, deactivating or scavenging free radicals before they attack cells (Sapakal *et al.*, 2008). Vitamin E and C are effective antioxidants and free radical scavengers. Therefore, the present study was carried out to investigate the potential protective effects of vitamin E and C individually and in combination against SO_2 gas induced hematological alterations in albino rats.

Materials and Methods

Experimental animals: Adult male wistar albino rats (average body wt. 150g) reared in polypropylene cages in standard conditions of temperature 25±0.5°C; relative humidity 60±5% and photoperiod 12h/d and were fed on pellet diet (Golden feed, New Delhi, India) and water *ad libitum*. Experimental rats were acclimated for one month prior to experiment.

Table 1. Values of TEC, TLC, Hb. Conc., PCV, ESR and red cell indices after SO_2 exposure and treatment with vitamin E and C in albino rats.

Parameters	Set-1 (5) Mean±S.Em.	Set-2 (5) Mean±S.Em.	Set-3 (5) Mean±S.Em.	Set-4 (5) Mean±S.Em.	Set-5 (5) Mean±S.Em.
TEC ($x10^{12}$/l)	7.77±.03	6.50±.38**	6.76±.51·	7.30±.35·	7.34±.20·
TLC ($x10^{9}$/l)	2.19±.05	2.76±.10***	2.40±.09·	2.27±.17·	2.30±.07·
Hb. Conc. (g/dl)	8.90±.18	7.86±.09***	9.02±.14·	9.20±.57·	9.70±.20*
PCV (percent)	41.0±1.04	37.0±1.09*	44.4±1.12·	42.0±.44·	45.0±1.26*
ESR (mm/hr)	2.8±.12	3.4±.18*	3.2±.12*	3.0±.22·	2.9±0.22·
MCV (fl)	57.96±2.48	53.11±3.02·	66.81±4.69·	58.21±3.67·	61.35±1.39·
MCH (pg)	12.49±.26	12.28±.76·	13.58±.97·	12.67±.49·	13.27±.58·
MCHC (g/dl)	21.73±.43	21.31±.63·	20.36±.59·	21.92±.78·	21.63±.86·

(5) = Number of albino rats
S.Em. = Standard error of mean

Significant difference from corresponding Set-1
·Non-significant (P>0.05)
*Significant (P<0.05)
**Highly significant (P<0.01)
***Very highly significant (P<0.001)

Experimental protocol: Experimental rats were grouped in 5 sets (1, 2, 3, 4 and 5) ontaining 5 rats each.

- Control set-(1): Exposed to ambient air for 1h/d for 28 days.
- Experimental set-(2): Exposed to 80 ppm SO_2 gas 1h/d for 28 days.
- Experimental set-(3): Exposed to 80 ppm SO_2 gas 1h/d with pre-exposure supplementation of vitamin E (2.5mg/rat) for 28 days.
- Experimental set-(4): Exposed to 80 ppm SO_2 gas 1h/d with pre-exposure supplementation of vitamin C (5mg/rat) for 28 days.
- Experimental set-(5): Exposed to 80 ppm SO_2 gas 1h/d with pre-exposure supplementation of vitamin E (2.5mg/rat) and C (5mg/rat) in combination for 28 days.

Hematological studies: Rats of each set were sacrificed after 28 days exposure to SO_2 gas and blood samples were collected directly from the ventricle of heart of the dissected rat with the help of sterilized disposable syringe fitted with hypodermic needles and were taken in double oxalate vials. Various hematological parameters were analyzed by following procedures in control and experimental groups of animals.

TEC and TLC were counted with the help of improved standard Neubauer hematocytometer (Dacie and Lewis, 1969). The hemoglobin concentration was estimated by the cyanmethaemoglobin method given by Mukherjee (1997). Packed cell volume and ESR were estimated by the Wintrobe's method (1981) and red cell indices (MCV, MCH and MCHC) were calculated by the (Wintrobe *et al.*, 1981).

The results were expressed as Mean±S.Em. by using students 't' test and statistical analysis were done by one-way ANOVA with the help of computer statistical program KpKy plot (version 3.0).

Results and Discussion

The results of the present study demonstrated the protective impact of vitamin E and C in reducing the toxic effects of SO_2 gas. At the end of 28 days exposure to 80 ppm SO_2 gas, there was a decrease in TEC, Hb. Conc., PCV and red cell indices, while increase in TLC and ESR

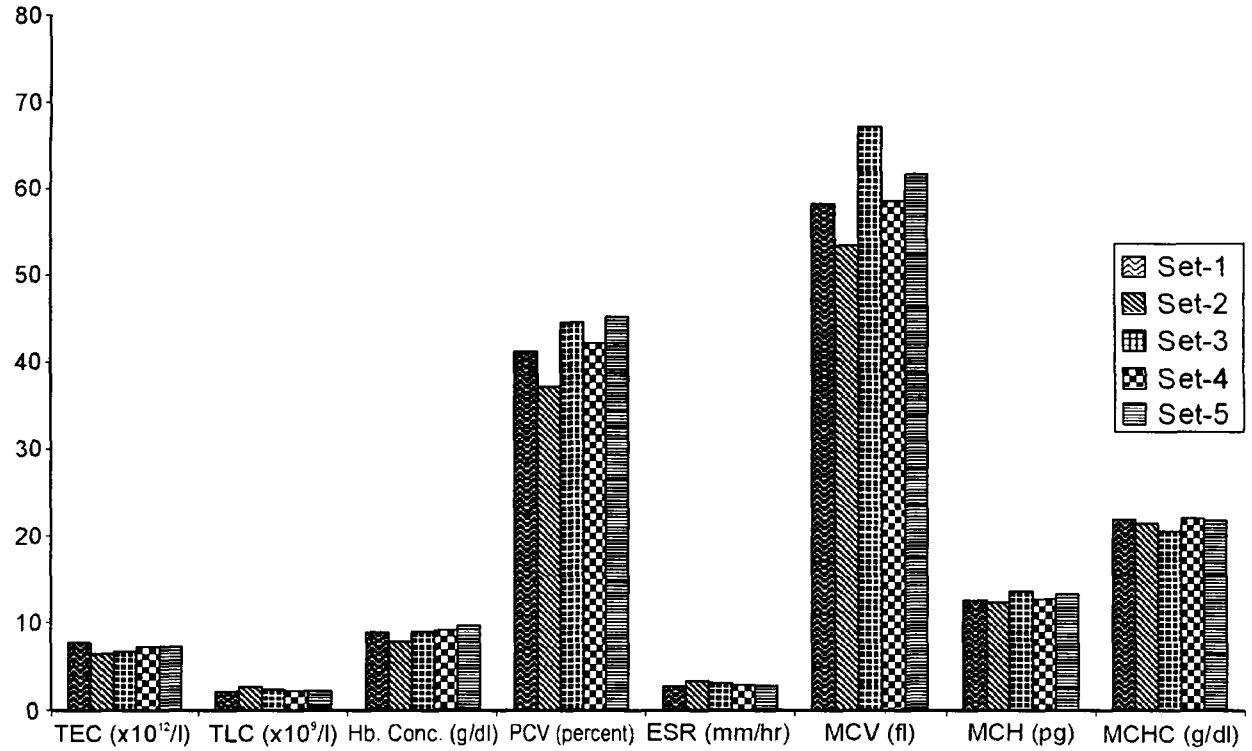

Fig. 1. Graph showing TEC, TLC, Hb. Conc., PCV, ESR and red cell indices after SO_2 exposure and treatment with vitamin E and C in albino rats

were observed. However, after supplementation of vitamin E and C individually and in combination increase in TEC, Hb. Conc, PCV and red cell indices, while decrease in TLC and ESR were noted (Table1, Fig.1).

In the present investigation, a significant alteration in hematological parameters is due to toxic action of SO_2 gas on haemopoietic tissues. It makes contact with the internal environment of the body through respiratory tract and binds to the methemoglobin pigments and lacking oxygen carrying capacity of the blood. SO_2 gas exposure may impose oxidative stress to the body (Smith *et al.*, 1995). The results are consistent with similar findings of Lal *et al.* (1993) who demonstrated that erythrocyte count and hemoglobin contents decreases after absorption to SO_2 in rats. The PCV is directly associated with the red cell count and hemoglobin concentration. Thus, lesser the RBC count, lesser the PCV (Guyton and Hall, 2002). Similar to the present findings Gorriz *et al.* (1996) stated that PCV decreased after exposure to SO_2 gas and air pollution in albino rats.

A decrease in the value of MCV, MCH and MCHC is indicative of the hypochromic microcytic anaemic condition and depend upon the value of total erythrocyte count, hemoglobin concentration and packed cell volume. Similar to the present findings, Baskurt (1988) observed decrease in MCHC after exposure to SO_2 gas. An increase in TLC is the result of inflammation due to irritant effects of SO_2 gas on respiratory tract, which causes leucocytosis. Haider (1985) and Gorriz *et al.* (1996) have also observed an increase in total leucocyte count after SO_2 gas exposure.

Alterations in hematological parameters were modulated after supplementation of vitamin E and C due to antioxidant defense mechanism. Vitamin E and C both help in heme synthesis and they work synergistically (Pant, 2004). Antioxidants protect the human body against damage by reactive oxygen species (Halliwell *et al.*, 1995). Some conditions may lead to

erythrocyte damage and hemolysis *in vivo* and *in vitro* by oxidative stress and these situations can be prevented by antioxidant treatment (Toker *et al.*, 1990; Jain, 1989; Regnoult *et al.*, 1993 and Etlik *et al.*, 1995). Administration of vitamin E and C in combination can reduce oxidative damage in erythrocyte induced by SO_2 gas inhalation in rats (Etlik *et al.*, 1997). Vitamin E and C treatment is effective in preventing inflammation like response (Senturk *et al.*, 2005). Lower the vitamin C concentration higher the serum levels of inflammatory responses in human.

The results of the present study reveal that supplementation of vitamin E and C in combination have more potential to mitigate SO_2 induced toxicity in comparison to vitamin E and C individually in albino rats.

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