IMPACT OF VITAMIN E AND C ON NITROGEN DIOXIDE GAS INDUCED ALTERATION IN BLOOD COAGULATION FACTORS OF MALE ALBINO RAT

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Abstract: In the present investigation, the effect of nitrogen dioxide exposure (50ppm) and role of vitamins E (2.5mg/rat) and vitamin C (5mg/rat) supplementation in combination on coagulation factors have been reported in male albino rats. An increase in coagulation factors viz. Prothombintime (PT), Partial thromboplastin time (PTT), Activated partial thromboplastin time (APTT), Thrombin time (TT) and platelet count (PLT), while decrease in fibrinogen deficiency plasma (FDP) level was reported after supplementation of vitamin E and C in comparison to nitrogen dioxide gas exposed rats. Present study suggests that administration of vitamin E and vitamin C in combination shows protective and beneficial effect to blood coagulation factors of albino rats against nitrogen dioxide gas toxicity.

Key words: NO2 gas, Vitamin (E+C), Coagulation factors

INTRODUCTION

NO2 is a chief constituent of photochemical smog in metropolitan cities. Important sources of NO2 are manufacture of explosions, anaerobic bacterial breakdown of nitrogenous compound, power plants, industrial installation and nitrogenous fertilizers. When NO2 exists in environment beyond its normal limit, it enters the human blood through lungs. NO2 is the known source of oxidants, involved in the pathogenesis of lungs and vascular system. The initial steps in the coagulation process involve clotting factors of the extrinsic pathways or the intrinsic pathways. PT, PTT, APTT, TT FDP and PLT count are useful tests for investigation of coagulation abnormalities caused by nitrogen dioxide gas toxicity. Supplementation of vitamin E and vitamin C parallel with gas may be beneficial to reduce the toxic effects generated by NO2 gas. It is likely that vitamins E and C act in a synergistic manner by vitamin E primarily oxidized to the tocopheroxyl radical and then reduced back to the tocopherol by vitamin C [1-3]. Thus, present study was designed to assess the impact of antioxidant supplementation on nitrogen dioxide gas induced coagulation disorders in male albino rats.

MATERIALS AND METHODS

Thirty adult healthy male albino rats of almost equal size and weight (100-125g) were selected for the present study. They were kept in polypropylene cages and were maintained under standard conditions of husbandry (12hrs light/dark, 22 ± 2°C room temperature and 50 ± 5% humidity). Rats were fed on Gold mohar Brand rat feed (Hindustan Unilever Ltd. India) and water was provided ad Libitum. The rats were randomly divided into control set (A) and
experimental sets (B & C) of ten rats each. The rats of control group were exposed to ambient air, while the experimental set B exposed to 50 ppm nitrogen dioxide gas alone and set C exposed to nitrogen dioxide gas with pre-exposure supplementation of vitamin E (2.5 mg/rat) and vitamin C (5 mg/rat) in combination by a gavage tube for one hour per day for 30 days. Nitrogen dioxide was generated in a nitrogen dioxide generator by Saltzman method [4]. Fumigation chamber was used for the exposure of rats. At the end of exposure period, rats were dissected and blood samples were taken in EDTA vials for platelet count and in centrifuge tubes containing 0.3 ml of citrate solution for separation of plasma. The blood samples were analyzed for each sample individually. PLT count was determined by Ree-Ecker method described by Mukherjee [5]. The PT was estimated by quick’s one stage method described by Mukherjee [5].

PTT and APTT were estimated by kit method described by Biggs [6]. TT was determined by commercially available method described by Mukherjee [5]. FDP was estimated by TULIP XL FDP kit method Colman et al. [7]. The data obtained from the observations were subjected to one way ANOVA test KP ky plot (ver.03)

**RESULT AND DISCUSSION**

The values of coagulation factors in control set (A) and experimental sets (B & C) for 30 days NO$_2$ exposure (50 ppm) and supplementation with vitamin (E+C) in combination. S. Em. = Standard Error of Mean, **Significant (P<0.05), (5) = Number of male albino rats, ***Highly–significant (P<0.01).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Exposure days</th>
<th>Set A (Control) Mean ± S.Em</th>
<th>Experimental Sets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Set B (Gas alone) Mean ± S.Em</td>
<td>Set C (Vit. C+E with gas) Mean ± S.Em.</td>
</tr>
<tr>
<td>PT (Sec)</td>
<td>30</td>
<td>17.9±0.30</td>
<td>16.8±0.44**</td>
</tr>
<tr>
<td>PTT (Sec)</td>
<td>30</td>
<td>37.3±0.31</td>
<td>36.6±0.38 **</td>
</tr>
<tr>
<td>APTT (Sec)</td>
<td>30</td>
<td>21.5±0.60</td>
<td>19.1±0.37 ***</td>
</tr>
<tr>
<td>TT (Sec)</td>
<td>30</td>
<td>18.3±0.36</td>
<td>14.1±0.21 ***</td>
</tr>
<tr>
<td>FDP (Sec)</td>
<td>30</td>
<td>9.8±0.07</td>
<td>10.9±0.12 ***</td>
</tr>
<tr>
<td>PLT Count x10$^9$/L</td>
<td>30</td>
<td>913±6.54</td>
<td>697±0.69 ***</td>
</tr>
</tbody>
</table>

Nitrogen dioxide gas induced decrease in PT, PTT, APTT, TT, and PLT count, while an increase in FDP level is accompanied with inflammation in albino rats. Oxidative stress in inflammatory cells release inflammatory cytokines has been linked to the activation of coagulation and decline in the activity of the defensive properties of the cell [8]. Platelet disorders are characterized by coagulation of blood and increased platelet consumption produce thrombocytopenia and a lack of adequate amount of other clotting factor also interferes with normal coagulation of blood [9]. All consistent with increased and quicker clotting also causes impairment in antioxidants defense mechanism.

After supplementation of vitamin E and vitamin C in combination, the increased level of PT, PTT, APTT, TT, and PLT count, while a decrease in FDP in male albino rats could be correlated with beneficial action against deleterious effects of nitrogen dioxide gas by scavenging the reactive oxygen species, thereby strengthening the antioxidants defense mechanism. Antioxidants vitamin E and vitamin C in combination act very quickly and decreased net intensity of inflammation caused by free radicals. Similar to the present findings, Brook et al. [10], reported that air pollution promotes plasma fibrinogen level and decreases plasma clotting factors in human which is linked with systematic inflammation and oxidative stress.

An elevation in plasma fibrinogen level and reduction in other clotting factors due to inflammation by the effect of air pollution have been reported by Lovett et al. [11], Pope et al. [12], Ruckeral [13] and Baccarelli et al. [14]. Vitamin E and vitamin C both decreases the effect of air pollution and protects against inflammatory disorders [15,16]. A protective role of vitamin E and vitamin C significantly reduces...
platelet aggregation and blood clotting disorders have also been reported by Senturk [17] and in a British daily report [18]. Present study suggests that supplementation of vitamin E and vitamin C in combination have an ameliorative impact against toxic action of nitrogen dioxide gas in male albino rats.

REFERENCES