Ameliorative Effect of Vitamin C And E Against The Toxicity of Nitrogen Dioxide Gas on Clotting Factors in Albino Rats

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ABSTRACT
In the present study, ameliorative effect of vitamin C and E against the nitrogen dioxide gas toxicity on blood plasma factors Fibrinogen deficiency plasma (FDP), Prothrombin time (PT), Partial thromboplastin time (PTT), Activated partial thromboplastin time (APTT), Thrombin time (TT) and calcium ions (Ca++) were studied in both the sexes of albino rats. A significant increase in plasma fibrinogen deficiency plasma level (FDP) and a significant decrease in prothrombin time (PT), partial thromboplastin time (PTT), activated partial thromboplastin time (APTT), thrombin time (TT) and calcium ions have been reported in both the sexes of albino rats after exposure to 50ppm nitrogen dioxide gas for 45 days for one hour per day.

A significant variation in plasma clotting factors is accompanied with inflammation and oxidative stress induced by toxic inhalation of nitrogen dioxide gas. Rats of equal size and weight (150-200gm) were kept in standard laboratory conditions and divided into three groups containing ten rats in each group. Group I is control set, group II and group III are experimental sets. Control set I was unexposed, set II was exposed to 50ppm nitrogen dioxide gas alone, while set III was exposed to 50ppm nitrogen dioxide gas along with supplementation of antioxidants vitamin C (5mg/rat) and vitamin E (2.5mg/rat) for one hour per day for 45 days. Results of present study shows that experimental set II shows disorders in clotting factors induced by nitrogen dioxide gas in comparison to control set I, while set III shows mitigation in disorder in clotting factors by supplementation of antioxidants vitamin C and E in comparison to experimental set II. Present study Reveal that toxicity of nitrogen dioxide gas have been attenuated by supplementation of vitamin C and E due to antioxidant defence mechanism in both the sexes of albino rats.

Keyword:- NO₂ Gas, Plasma Clotting Factors, Albino Rats and Vitamin C and E.

INTRODUCTION
Now a days so many air pollutants are present in the environment and causing so many health hazards day by day problems of air pollution increasing by leaps and bounds. Air pollutants include many gases among them nitrogen dioxide gas is one of the major air pollutants, these are not considered as air pollutant while they did not cross their normal limit. After crossing their normal limit they start causing harm to human beings. Air pollutants released from different sources like automobiles engines, chemical industries and thermal power plant. Industries release their waste directly in environment from automobiles so many toxic gases are released in which nitrogen dioxide is also a harmful gas and reaches to human beings through inhalation from environment by the nasal passage and it interacts with blood stream induced changes in
blood parameters and causes so many changes by changing their normal values. In blood clotting factors viz.(FDP) fibrinogen deficiency plasma,(PT) prothrombin time,(PTT) partial prothrombin time,(APTT) activated partial thromboplastin time,(TT) thrombin time and calcium ions(Ca++) have been altered by the inhalation of nitrogen dioxide gas, Which induced oxidative stress and inflammatory conditions. The two main functions of the coagulation system are to produce thrombin which aids the physiological action of platelets in haemostasis and form a fibrin network. Each and every clotting factors has its own importance in the process of blood coagulation. Important clotting factors include calcium ions and 13 different proteins, called proenzymes, that can be converted to active enzymes. During the coagulation phase, the proenzyme interacts, and after the conversion of one proenzyme creates an enzyme that activate a second proenzyme, and so on, in a chain reaction. To overcome with these conditions antioxidants vitamin c and e have been given parallel with nitrogen dioxide gas. Vitamin C and E shows a potential role in mitigating oxidative stress and inflammatory conditions in both the sexes of albino rats.

MATERIAL AND METHODS:
Thirty adult male and female albino rats of equal size and weight (150-200gm) were kept in polypropylene cages in standard conditions of temperature 25± 0.5, relative humidity 60±5% and photoperiod of 12 hr/day and were fed on pellet diet(Golden feed,New Delhi,India) and water was given at ad libitum Experimental animals were acclimatized for two weeks prior to experiment .All the experiment were carried out as per guideline of Institutional Ethical Committee.

Selection of Experimental Gas:-
Nitrogen dioxide was selected for the present study and prepared by the method of Saltzman21 and modified by Levaggi11.

Selection of Antioxidants:-
Evion drops as vitamin E (Tocopherol acetate2.5mg/rat) and vitamin C as (Ascorbic acid 5mg/rat) from Merck company, Aurangabad were used as antioxidants.

Experimental Protocol:-
The albino rats were grouped in to three sets. Group I for Control set and group II and III were for Experimental sets containing 10 rats each.
Control Set I:-Unexposed
Experimental Set II: - Exposed to 50ppm nitrogen dioxide gas for one hour per day for 45 days.
Experimental Set III:-Exposed to 50ppm nitrogen dioxide gas with supplementation of vitamin C (5mg/rat) and vitamin E (2.5mg/rat) for one hour per day for 45 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 whole body exposure for one hour per day for 45 days.

Sample Collection:-
Fifteen male and fifteen female albino rats of control set I(),experimental Set II and experimental set III were sacrificed after 45 days. Blood samples were collected directly from the ventricles of the heart of dissected rat with the help of sterilized disposable syringe fitted with hypodermic needles and taken in centrifuge tubes containing 0.3 ml of citrate solution for the separation of plasma. Plain sterilized centrifuge were used for the separation of serum (for estimation of calcium ions).

METHODS
Prothrombin Time (PT)
The prothrombin time was by quick’s one stage method described by Mukherjee(1997).
Partial Thromboplastin Time (PTT)
Partial thromboplastin time was estimated by kit method described by Biggs\textsuperscript{7}.

Activated Partial Thromboplastin Time (APTT)
The activated partial thromboplastin time was estimated by kit method described by Biggs (1972).

Thrombin Time (TT)
Thrombin time was determined by commercially available method described by Mukherjee (1997).

Fibrinogen Deficiency Plasma (FDP)
Fibrinogen deficiency plasma was estimated by TULIP XL FDP kit method given by Colman et al.\textsuperscript{10}.

Calcium Ions (CA++)
Serum calcium ions (ca++) was determined by Span diagnostic method, O.C.P.C. Method described by Lorentz (1982).

RESULTS AND DISCUSSION

In the present study, a significant increase in plasma fibrinogen deficiency (FDP) level and a significant decrease in prothrombin time (PT), partial thromboplastin time (PTT), Activated partial thromboplastin time (APTT), thrombin time (TT) and calcium ion (Ca\textsuperscript{++}) have been reported in both the sexes of albino rat after exposure to nitrogen dioxide gas. A significant variation in plasma clotting factors is accompanied with inflammation induced by toxic inhalation of nitrogen dioxide gas. Hypofibrinogenemia, a lack of circulating fibrinogen is associated with inflammation that is a direct result of changes in behavior of local blood vessels. As a result of damage to the blood vessel walls leads to a series of enzyme reactions that result in large quantities of thrombin, is a main clotting enzyme being formed. It acts on the fibrinogen in tissue fluid and plasma to produce insoluble strands of fibrin, which are laid down in inflamed tissue and capillaries and form an effective barrier to the tissue injury (Tizard\textsuperscript{23}). A calcium (Ca\textsuperscript{++}) deficiency can produce coagulation disorders (Pennington, 1978). Pekkanen et al.\textsuperscript{17} have found that an increment in plasma fibrinogen deficiency level possibly associated with inflammation induced by air pollution in male and female office worker. Schwartz\textsuperscript{22} has been noted that increased plasma fibrinogen deficiency is associated with inflammation with the effect of air pollution in humans.

Present findings are agreement with the findings of Brook et al.\textsuperscript{6} who have reported that air pollution promotes plasma fibrinogen deficiency level and decreased plasma clotting factors in human which is linked with systematic inflammation and oxidative stress. Nel\textsuperscript{15} has also stated that air pollution enhanced the blood clotting with increase plasma fibrinogen deficiency concentration with defective clotting factors in volunteers. Rivero et al.\textsuperscript{20} have observed an elevation of fibrinogen deficiency level depend up on the concentration of air pollution in male and female rats may be associated with systematic inflammation. Similar observations also made by Ruckerl et al.\textsuperscript{19} who have reported an elevation in plasma fibrinogen deficiency level and a decrease in clotting factors associated with systematic inflammation in patients by the effect of air pollution. Similarly, Baccarelli et al.\textsuperscript{5} have found that an increase in plasma fibrinogen deficiency level was associated with decreased clotting factors due to inflammation caused by air pollution in humans.

In support of present findings, Stone (2000) have reported that increment in deficiency of fibrinogen concentration by PM effect which induces inflammation in adults, while an increase in plasma fibrinogen deficiency level with decrease in clotting factor due to inflammatory response have been reported by Nemmar et al.\textsuperscript{15} in rat by diesel exhaust\textsuperscript{16}. Similar observations regarding increase in plasma fibrinogen deficiency level and decrease in clotting factors due to inflammatory responses are also given by Riediker et al. (2004) with particulate matter exposure in healthy men, while Reed et al.\textsuperscript{18} in male and female rats due to inflammatory responses caused by environmental level of diesel exhaust. In add support to the present findings Blomberg et al.\textsuperscript{5} have reported increased fibrinogen deficiency level and decreased clotting factors due to inflammation by diesel exhaust nanoparticles in individuals. Burton\textsuperscript{8} have also observed an increase
in fibrinogen deficiency concentration and decrease in PT and PTT are consistent with increased in clotting time by the exposure of PM due to inflammation in mice. Supporting to the present findings, Mutlu *et al.* (2007) have also found out that increase in plasma fibrinogen deficiency concentration and decrease in PT, PTT, APTT and TT accompanied with inflammatory action of PM exposure in male and female rats. In the present study, the reduction in toxic effect of nitrogen dioxide gas on the blood parameters related to clotting disorders in both the sexes of albino rat after supplementation of vitamin (C+E) in combination as synthetic antioxidants and with dietary antioxidants as natural antioxidants, 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). Antioxidants vitamin C and E in combination act 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. As there is evidence that vitamin C+E both work together synergistically and prevent cell destruction (Beyor, Chen and Tappel, Lass and Shoal).

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 who stated that toxic effect of petroleum may be reduced by supplementation of vitamin C+E by playing a protective role.

**Table 1:** Blood plasma clotting factors in both the sexes of albino rats after 45 days exposure (50ppm) and supplementation with vitamin (C+E)

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>CONTROL SET-1</th>
<th>EXPERIMENTAL SET –II (ONLY GAS 50PPM)</th>
<th>EXPERIMENTAL SET –III (GAS 50PPM WITH VITAMIN C+E)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MALE</td>
<td>FEMALE</td>
<td>MALE</td>
</tr>
<tr>
<td>PT(Sec)</td>
<td>16.8-18.9</td>
<td>14.8-16.8</td>
<td>15.2-17.2</td>
</tr>
<tr>
<td></td>
<td>17.6±0.37</td>
<td>16.0±0.44</td>
<td>16.1±0.40 ↓</td>
</tr>
<tr>
<td>PTT(Sec)</td>
<td>36.2-38.2</td>
<td>34.4-37.6</td>
<td>35.8-36.8</td>
</tr>
<tr>
<td></td>
<td>37.0±0.36</td>
<td>36.0±0.54</td>
<td>36.1±0.17 ↓</td>
</tr>
<tr>
<td>APTT(Sec)</td>
<td>20-22.8</td>
<td>19.6-20.7</td>
<td>18.2-20.4</td>
</tr>
<tr>
<td></td>
<td>21.6±0.50</td>
<td>20.2±0.23</td>
<td>19.2±0.30 ↓</td>
</tr>
<tr>
<td>TT(Sec)</td>
<td>17.4-19.0</td>
<td>16.2-17.8</td>
<td>13.0-19.0</td>
</tr>
<tr>
<td></td>
<td>18.2±0.28</td>
<td>16.6±0.43</td>
<td>18.0±0.28 ↓</td>
</tr>
<tr>
<td>FDP(Sec)</td>
<td>8.9-10.0</td>
<td>7.9-9.9</td>
<td>12.3-14.0</td>
</tr>
<tr>
<td></td>
<td>9.6±0.19</td>
<td>8.6±0.34</td>
<td>13.1±0.32†</td>
</tr>
<tr>
<td>Ca++(mg/dl)</td>
<td>10.0-11.4</td>
<td>10.2-11.2</td>
<td>9.7-10.2</td>
</tr>
<tr>
<td></td>
<td>11.0±0.11</td>
<td>10.7±0.17</td>
<td>9.9±0.04†</td>
</tr>
</tbody>
</table>

ppm = parts per million
(5) = Number of albino rats

- **Non–significant (P>0.05)**
- **Decrease**
- **Significant (P<0.05)**
- **Increase**

**M = Male, F = Female**
**S.Em. = Standard Error of mean**

**CONCLUSION**

The result of present study shows that supplementation of vitamin C and E in combination have a pronounced ameliorative effect against the toxic effect of nitrogen dioxide gas in both the sexes of albino rat.
REFERENCES.


