BIOCHEMICAL AND HAEMATOLOGICAL ALTERATIONS IN RATS FOLLOWING ACUTE INHALATION EXPOSURE OF GASOLINE, METHANOL AND GASOLINE:METHANOL BLEND

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Abstract: Gasoline is a mixture of aliphatic and aromatic hydrocarbons and used as a motor fuel. Current trend to add methanol to gasoline (10:90, v/v) warrants toxicological evaluation of gasoline:methanol blend. The present study was designed to evaluate the effect of gasoline:methanol blend vapours exposure which caused a significant increase in acid phosphatase activities in broncho-alveolar lavage fluid (BALF) and lung as well as LDH activity in the serum of rat. The proteins content was increased significantly in the lung and insignificant in serum as compared to control. The study showed rise in haemoglobin, haematocrit, MCV and lymphocytes and decrease in MCHC, platelets, neutrophils and eosinophil count. All these changes were significant. Nevertheless, methanol exposure alone did not show significant changes. The above findings indicate inflammatory reaction along with increased phagocytic activity in the lungs of rats exposed to gasoline and gasoline:methanol blend through inhalation route of exposure.

Key words: Gasoline, Methanol, Inhalation toxicity, Rat

INTRODUCTION

The New Energy Development Organization (NEDO) in Japan and Health Effects Institute (HEI), USA has initiated programmes to evaluate the human health following exposure to automotive gasoline:methanol blend. Toxicity studies in rats following continuous inhalation of methanol-fueled engine exhaust indicated concentration and time dependent changes in blood and target organs. An increase in carboxy-haemoglobin in red blood cells and formaldehyde in plasma; increased serum albumin and decrease in activity of alkaline phosphatase and cytochrome P 450 contents in lungs have been reported [1]. Gupta et al. [2] observed that subcutaneous administration of gasoline:methanol blend in adult male druekery rats for 28 days caused a decrease in serum proteins particularly albumin and free sulfahydryl content of kidney.

The studies of Gupta et al. [2] and Poon et al. [3] indicate increased absorption of gasoline in presence of methanol. It is apparent, therefore, that commercialization of gasoline:methanol blend may involve higher risk of toxicity as compared to gasoline alone. Our previous study indicated a mild thickening of alveolar wall with infiltration of inflammatory cells and emphysema in the lungs of rats exposed to acute dose of gasoline:methanol blend vapour [4]. The present study extents the investigation on some biochemical parameters in the lung and serum of rat in same experimental conditions.

MATERIALS AND METHODS

Chemicals: Gasoline was procured from an authorized outlet of Indian Oil Corporation Ltd. All other solvents and chemicals used in the present study
were of analytical grade procured from Sigma, Aldrich (USA), Qualigens, or S.D. Fine Chemicals (Mumbai, India).

Experimental animals: Colony bred healthy, adult, Wistar strain rats (100 to 125g) obtained from breeding facility of Jai Research Foundation, Valvada (India) were used in the present experiments. They were fed with Amrut brand laboratory rat pellet feed (Manufactured by M/s Nav Maharashtra Chakan Oil Mills Ltd., Pune, India) and aquaguard filtered water ad libitum. The animals were maintained under 12 h light/dark cycles at 22 ± 3°C.

Experimental design: The present study was carried out following the guidelines of OECD [5] with a specially designed nose only inhalation exposure chamber. The animals were randomly divided into four groups (10 animals, 5 male and 5 female in each group) and caged separately (see experimental protocol in Table 1).

Generation and characterization of exposure atmospheres: To study the repeated dose inhalation toxicity of gasoline, methanol and its blend (90:10, v/v) in rats, the experiments were carried out using inhalation equipment (nose only exposure) of Bio-Tox Instrumentations International, New Delhi, India.

The gasoline, methanol or gasoline:methanol blend was loaded into 60 ml disposable syringe which was then positioned on a continuous infusion syringe pump (manufactured by B. Braun Melsungen AG, Germany). The content of the syringe was infused at the rate of 30 ml/h into the nebulizer where vapour aerosol was formed and distributed into the respective inhalation chamber. The rate of air inflow and outflow was maintained at 10 and 11 L/min, respectively. Each rat was restrained in a single transparent polyacrylic rat exposure tube with an adjustable unit. Observations during the inhalation experiment were made. The animals were exposed continuously for four hours to test vapours only after an equilibration period of 30 min [6].

The actual concentration at the breathing zone level was determined gravimetrically. The open phase sampler was used to assess the breathing zone concentration where pre-weighed glass microfibre filters (Whatman GF/A) and a backup collection of polyurethane foam packs were used [7]. The test vapour samples were collected for five minutes at the rate of 2 L/min. Thereafter, the open phase sampler was disassembled and the glass microfiber filters and polyurethane foam packs were weighed (post-weight) to determine the breathing zone concentration of test sample in the inhalation chamber.

Collection of blood, bronchoalveolar lavage fluid (BALF) and lung: Prior to sacrifice, blood samples were collected by puncturing the orbital sinus with the help of a fine capillary tube under ether anesthesia. Around 0.5 ml of blood was collected in vials containing EDTA for haematological analysis. One drop of blood was taken on clean glass slide, spread and stained with Leishman’s stain for differential leucocyte count. Approximately 2 to 3 ml of blood was collected from each rat in clean centrifuged tubes for serum separation. The blood was allowed to clot at room temperature and the serum was separated by centrifuging it at 3000 rpm for 15 min. For haematological analysis RBC (10⁶/µl), WBC (10⁳/µl) and platelet count (10³/µl), haemoglobin (g/dl), HCT (%), MCV (fl), MCH (pg), MCHC (g/dl), Sysmex K 1000, manufactured by Tao Medical Electronics Co. Ltd., Kobe, Japan, was used and serum parameters were performed using Erbachem-5 plus, supplied by Transasia Bio-Medicals Pvt. Ltd., Mumbai, India.

The rats were sacrificed by an overdose of carbon dioxide at different time intervals viz., 16, 24, 48 and 72 h after inhalation exposure. The bronchoalveolar lavage fluid was collected by the method of

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Dose of treatment</th>
<th>Treatment (concentration)</th>
<th>Duration of treatment (hours)</th>
<th>Time of autopsy (hours after treatment)</th>
<th>Number of animals exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle treated control (air only 10 L/min)</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>II</td>
<td>Gasoline alone</td>
<td>30 ml/h, 548.3 mg/m³ air</td>
<td>4</td>
<td>16, 24, 48 and 72</td>
<td>40</td>
</tr>
<tr>
<td>III</td>
<td>Methanol alone</td>
<td>30 ml/h, 553.6 mg/m³ air</td>
<td>4</td>
<td>16, 24, 48 and 72</td>
<td>40</td>
</tr>
<tr>
<td>IV</td>
<td>Gasoline: methanol blend</td>
<td>30 ml/h, 531.5 mg/m³ air</td>
<td>4</td>
<td>16, 24, 48 and 72</td>
<td>40</td>
</tr>
</tbody>
</table>
Henderson [8] as adopted by Gupta et al. [9]. Collected BALF was centrifuged at 2000 rpm for 10 min to sediment the cells. The supernatant was separated and used for various biochemical analysis.

The activity of acid phosphatase in BALF and lung (µmol. p-nitrophenol generated/100 ml/30 min) was estimated by the method of Bessey et al. [10], LDH activity in serum (IU/L) was assayed using the method of Henry et al. [11] and concentration of proteins in lungs (µg/100 mg tissue) and serum (g/dl) were estimated by the method of Lowry et al. [12].

**Data evaluation:** The data was subjected to Student’s test for statistical analysis [13].

**RESULTS**

The mean breathing zone concentration of gasoline, methanol and gasoline:methanol blend in the inhalation chamber measured by gravimetric method and was found to be 548.3, 553.6 and 531.5 mg/m³ of air respectively. No mortality in control and treated rats was observed during the course of study.

Table 2 shows the effect of gasoline, methanol and gasoline:methanol vapour exposure on biochemical changes in the lung, BALF and serum of rats. As compared to control, significantly higher acid phosphatase activity was found in BALF and lung tissue at 16, 24 and 48 h after gasoline and gasoline:methanol vapour exposure but normalcy was regained at 72 h. Proteins content and LDH activity were significantly higher in the lung and serum respectively after 72 h exposure. However, methanol exposure alone did not cause any significant alterations and the concentration of serum proteins remained unchanged in all the animal groups.

The effect of gasoline, methanol and gasoline:methanol vapour on haematological parameters is shown in table 3. The haematological parameters is shown in table 3. The haematological

<table>
<thead>
<tr>
<th>Parameters</th>
<th>16 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>7.29±0.18</td>
<td>7.41±0.25</td>
<td>7.31±0.15</td>
<td>7.12±0.10</td>
</tr>
<tr>
<td>WBC</td>
<td>6.73±0.58</td>
<td>6.87±0.62</td>
<td>6.85±0.53</td>
<td>7.69±0.69</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>13.38±0.29</td>
<td>14.29±0.45</td>
<td>13.27±0.42</td>
<td>14.16±0.54</td>
</tr>
<tr>
<td>HCT</td>
<td>37.58±0.70</td>
<td>43.38±0.85</td>
<td>37.18±0.58</td>
<td>42.13±0.58</td>
</tr>
<tr>
<td>MCV</td>
<td>51.61±0.70</td>
<td>60.39±0.83</td>
<td>51.62±0.85</td>
<td>59.58±0.85</td>
</tr>
<tr>
<td>MCH</td>
<td>18.43±0.20</td>
<td>18.50±0.21</td>
<td>18.35±0.19</td>
<td>18.35±0.13</td>
</tr>
<tr>
<td>MCHC</td>
<td>35.89±0.22</td>
<td>31.82±0.72</td>
<td>35.23±0.19</td>
<td>31.10±0.42</td>
</tr>
<tr>
<td>Platelet count</td>
<td>383.00±0.90</td>
<td>755.42±1.00</td>
<td>871.60±1.20</td>
<td>768.80±1.30</td>
</tr>
</tbody>
</table>

Values are mean and SEM, n = 10; As compared to control (Group I): Significant at the level, *P<0.05, **P<0.01, ***P<0.001; Group I control, Group II, III and IV were exposed to 30 ml/h gasoline (548.3 mg/m³ air), methanol (553.6 mg/m³ air) and gasoline:methanol blend (531.5 mg/m³ air) vapour for 4 h and sacrificed at 16, 24, 48 and 72 h interval.

**Table 2:** Effect of acute gasoline, methanol and gasoline: methanol vapour on some biochemical parameters in BALF, lung and serum of rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>16 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid phosphatase in BALF</td>
<td>3.86±0.11</td>
<td>8.09±0.22</td>
<td>3.82±0.19</td>
<td>8.08±0.27</td>
</tr>
<tr>
<td>Acid phosphatase in lungs</td>
<td>9.60±0.89</td>
<td>20.44±0.40</td>
<td>9.52±0.32</td>
<td>22.50±0.24</td>
</tr>
<tr>
<td>Proteins in lungs</td>
<td>23.64±1.75</td>
<td>35.52±1.62</td>
<td>24.37±1.40</td>
<td>34.40±1.08</td>
</tr>
<tr>
<td>Serum proteins</td>
<td>6.60±0.09</td>
<td>6.54±0.17</td>
<td>6.66±0.10</td>
<td>6.66±0.10</td>
</tr>
<tr>
<td>Serum LDH</td>
<td>1373±129.3</td>
<td>2013.3±138.6</td>
<td>1287.1±119.1</td>
<td>2121.3±114.1</td>
</tr>
</tbody>
</table>

Effect of acute gasoline, methanol and gasoline: methanol vapour on haematological parameters of rats.
analysis revealed significant rise in haematocrit (HCT) and mean corpuscular volume (MCV), together with significant reduction in mean corpuscular haemoglobin concentration (MCHC) in exposed rats. The significant increase in mean corpuscular haemoglobin (MCH) was noticed at 48 and 72 h sampling time both in gasoline and gasoline:methanol exposed rats. Significantly higher haemoglobin concentration and lower platelets count up to 72 h sampling time was recorded. However, changes in RBC count were insignificant.

The differential leucocyte count of various groups is shown in table 4. The lymphocyte count was higher but neutrophil and eosinophil counts were reduced up to 72 h sampling time both in gasoline and gasoline:methanol vapours exposed rats as compared to control. However, acute changes were not observed in the methanol alone exposed rats.

**DISCUSSION**

It has been reported that composition and toxicity of gasoline varies depending on nature, processing and the source of crude oil, blending conditions, geographical location as well as seasonal variations [2]. The effect of these variables either singly or in combination may diversely affect the toxicity of gasoline. The significantly higher activity of acid phosphatase in BALF and lung along with increased proteins content in lung of gasoline and gasoline:methanol treated rats is the result of cellular damage in lungs. The increased LDH activity during these treatments also indicates early damage in lung [14]. The mechanism may be similar to that of activation of carbon tetrachloride by pulmonary Clara cells [15] as well as type I and type II alveolar epithelial and pulmonary endothelial cells [16-18]. Hendersen [8] advocated measurement of several enzymatic profiles in lavage fluid as a sensitive indicator of lung damage. Gupta et al. [2] also reported enhanced alkaline phosphatase activity in the lung and BALF after administration of gasoline and gasoline:methanol blend in rats.

MCV, MCH, haemoglobin content and haematocrit were significantly increased in rats exposed to acute gasoline and gasoline:methanol vapours, while RBC count remained unchanged. Poon et al. [3] also found increased haemoglobin concentration in rats exposed to gasoline vapours. The increased haemoconcentration occurs in shock, which may be associated with stress while, haematocrit also increases with primary polycythemia caused by increased erythropoiesis in pulmonary diseases [19]. An increase in MCV might be due to the release of newly generated erythrocytes and reduction in MCHC could be due to significant rise in MCV in exposed rats.

The alterations caused by acute inhalation exposure of gasoline and gasoline:methanol vapour on differential leucocyte count confirms acute inflammation along with the increased phagocytic activity. A decrease in neutrophils (neutropenia) often occurs following acute haemorrhages caused by acute infection or inflammation. Reduction in platelet count might be due to haemorrhagic disorders, the most common of which is leakage of blood from capillaries after a minor injury. All the above findings indicate the acute inflammatory process with increased phagocytic activity.

It is evident that most of the observed changes are the effects of gasoline vapours without any interaction with methanol. In the presence of methanol there could be a possibility of greater absorption of gasoline during exposure and the effects may be more severe after long-term inhalation. The study reveals that an understanding of the mechanism of gasoline-induced...
pulmonary toxicity and health risk assessment should be taken into consideration before advocating gasoline:methanol mixtures as a fuel for vehicles.

ACKNOWLEDGEMENTS

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REFERENCES