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# DNA damage in viscose factory workers occupationally exposed to carbon di-sulfide using buccal cell comet assay

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## Abstract

**Aim:** The most important industrial use of carbon disulfide ($CS_2$) has been in the fabrication of regenerated cellulose rayon by the viscose process and cellophane. $CS_2$ leads to increased frequency of chromosomal aberrations in workers with occupational exposure to $CS_2$. **Methods:** In the present study, the DNA damage was analyzed by using buccal cell comet assay for 30 viscose plant workers who are occupationally exposed to $CS_2$ and 30 healthy individuals. Both groups were classified as smokers and non-smokers and only the experimental subjects were classified based on the exposure period. The data were analyzed statistically by the Student's t-test. **Results:** The results of this study showed increased levels of DNA damage among viscose plant workers. **Conclusion:** The habit of cigarette smoking among the viscose workers had a synergistic effect on inducing DNA damage.

**Keywords:** carbon di-sulphide, DNA damage, smoking, buccal cell comet assay.

## Introduction

Carbon disulfide ($CS_2$) is an important industrial liquid organic solvent, which is mainly used to treat alkali cellulose in the viscose process (a source of rayon and cellophane). $CS_2$ may react with chlorine in the presence of a catalyst to form carbon tetrachloride also toxicants. In past years, studies have shown different potential cytotoxic effects of $CS_2$ on mammals[1-2]. Acute and subacute poisoning appear due to exposure to $CS_2$ concentrations of 500-3000 mg/m$^3$ and are predominantly characterized by neurological and psychiatric symptoms, gastrointestinal disturbances and genderual disorders[3-10], whereas exposure to $CS_2$ concentrations above 5000 mg/m$^3$ may induce coma or even death[11]. The toxic effects of $CS_2$ on experimental animals[12-15] have been extensively demonstrated and epidemiological studies on $CS_2$ exposure among workers in viscose rayon plants have been also reviewed, including studies of ischemic heart disease (IHD) mortality for workers in the viscose rayon industry[16-18].

Comprehensive testing of the mutagenic potential of $CS_2$ has been performed on several types of bacteria (Ames test) and Drosophila, with no positive results[19]. Further studies on Salmonella typhimurium, Drosophila, human fibroblasts cultures, human blood leucocytes and rats have been inconclusive[20]. Numerous studies have shown elevated standard mortality ratios (SMRs) for workers occupationally exposed to $CS_2$. Nonetheless, there are no reports available for $CS_2$ that provide strong evidence of genotoxic effects on DNA. Since the buccal epithelium provides an alternative source of tissue for monitoring human exposure to occupational and environmental genotoxins[21]. The present study was carried out to assess, using buccal cell comet assay, the genotoxicity among viscose plant workers who are occupationally exposed to $CS_2$.

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## Materials and methods

### Subject recruitment

The study subjects were 30 viscose plant workers and 30 healthy individuals as controls who were selected from various cities of southern India between August 2008 and January 2009. Prior to enrollment in the the study, all subjects gave written informed consent. A questionnaire was used to collect information on gender, age, duration of exposure, use of protective masks, general health status, smoking habits and exposure to drugs for each experimental and control subject. There were 12 smokers and 18 non-smokers in each group. The average cigarette consumption of smokers in both groups was nearly 13.4 ± 3.0 (mean±standard deviation) cigarettes/day. Ethical approval for this study was granted by the Ethics Committee of Bharathiar University.

### Sample collection

Buccal cells were collected from subjects by oral brushing. Prior to brushing, subjects washed their mouth with normal saline to avoid the interference of mucus. Collected samples were taken in cold phosphate buffer saline (PBS) and cells were allowed to pellet down. The cells were then resuspended in 300 ìL PBS and 50 ìL of cell suspension were taken for comet assay.

### Comet assay

Comet assay was performed under alkaline conditions by using a standard protocol[22] with some modifications[23]. Cells were embedded in low melting point agarose on glass slide precoated with 1% normal agarose. After solidification of gel, the slide was submerged into cool lysis solution [2.5M NaCl, 100 mM EDTA, 10 mM Tris (pH 10.0), 1% LSS lauryl sarcosine sodium salt to which 10% DMSO, 1% Triton X-100 were freshly added] and kept overnight at 4°C. The slides were then placed on the horizontal electrophoresis unit filled with freshly prepared alkaline electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH 13) for 30 min and then subjected to electrophoresis at 25V/ 300mA for 40 min. After electrophoresis the slides were neutralized for ~60 min in 0.4 M Tris/HCl, pH 7.5 on ice, followed by staining in ethidium bromide (stock concentration 25 ìg/mL in distilled water) and mounting on glycerol. All steps were performed on ice to prevent the removal of thin agarose gel layer from the slide. The stained slides were examined under Nikon fluorescent microscope with a 580nm emission filter.

### Statistical analysis

Results are expressed as mean ± standard deviation. Student's t-test was performed to compare the DNA damage levels between the experimental and controls. Statistically significant levels were considered at $p < 0.05$.

## Results

The subjects were selected from viscose plant workers who are occupationally exposed to CS2. The tail movement of comets observed in the buccal cells of experimental and controls are given in Table 1. In the control group, the percentage of DNA damage observed among smokers was higher than that observed among non-smokers, though without statistically significant data was observed except for the 3 subjects aged 26-35 years. An age-related increase in DNA damage was observed in both control and experimental subjects.

Experimental subjects over 46 years of age showed maximum DNA damage (25.3±0.3%). Significant increase ($p < 0.05$) of DNA damage percentage was identified in most individuals of the experimental groups when compared to the control subjects. An increased level of DNA damage was observed in the viscose plant workers with smoking habits when compared to smoking controls and nonsmoking viscose plant workers. To determine the effect of duration of exposure to $CS_2$ on DNA damage, the workers were divided into 2 groups depending on whether they had less than 10 years of exposure or more than 10 years of exposure (Table 2). No statistically significant difference in DNA damage was observed with increased duration of exposure to $CS_2$ (Table 2).

## Discussion

Mutagenesis is involved in the pathogenesis of many neoplasias. Occupational exposure may contribute to the development of pernicious illnesses, many times through mechanisms that involve genotoxic changes. Continuous efforts have been made to identify genotoxic agents, to determine conditions of harmful exposure and to monitor populations that are excessively exposed[24-25].

Table 1 - Classes of comets and percentage of DNA damage among the control and experimental subjects

| Subjects | Groups | Number of subjects | Percentage of DNA damage |
|---|---|---|---|
| Control smokers | <25 | 5 | 6.3 ± 0.1 |
| | 26-35 years | 3 | 8.1 ± 0.3* |
| | 36-45 years | 2 | 14.0 ± 0.3* |
| | 46-55 years | 2 | 16.6 ± 0.2 |
| Control non-smokers | <25 | 7 | 7.2 ± 0.4 |
| | 26-35 years | 4 | 8.6 ± 0.2 |
| | 36-45 years | 3 | 11.8 ± 1.2 |
| | 46-55 years | 4 | 14.1 ± 0.4 |
| Experimental smokers | <25 | 5 | 14.1 ± 0.6 |
| | 26-35 years | 3 | 16.1 ± 0.5** |
| | 36-45 years | 2 | 21.2 ± 0.1 |
| | 46-55 years | 2 | 25.3 ± 0.3** |
| Experimental non smokers | <25 | 7 | 11.4 ± 0.7* |
| | 26-35 years | 4 | 14.4 ± 0.4* |
| | 36-45 years | 3 | 18.5 ± 1.4* |
| | 46-55 years | 4 | 20.2 ± 0.3 |

* $p < 0.05$ compared with non-smoking control subjects; ** $p < 0.05$ compared with smoking control subjects.

Table 2 - Percentage of DNA damage according to duration of exposure in experimental subjects

| Exposure period | Number of subjects | Percentage of DNA damage |
|---|---|---|
| <10 years | 12 | 12.9 ± 3.1 |
| > 20 years | 18 | 13.27 ± 4.7 |

The present study was designed to assess the DNA damage among viscose plant workers who are occupationally exposed to $CS_2$. Comet assay is a valuable method for detection of occupational and environmental exposures to genotoxicants, and it can be used as a tool in risk assessment for hazard characterization[26-27], air pollution[28], cigarette smoking[29] and various in vitro and in vivo studies[30].

In the present investigation, a notable DNA damage was observed among the healthy controls. It is due to the assay being widely used in studying DNA damage in healthy individuals[31] and day to day variation in buccal epithelial cell strand breaks[32]. There was significant difference between experimental and control subjects who are occupationally exposed to $CS_2$. In past years, $CS_2$ concentrations in viscose rayon plants averaged about 250 mg/m$^3$; they were subsequently reduced to 50-150 mg/m$^3$ and more recently exposure levels of $CS_2$ are mostly below 31 mg/m$^3$[3,33]. A report on hypospermia, asthenospermia and teratospermia in young workers exposed to 40-80 mg/m$^3$ of $CS_2$ confirmed gonadal injury[34]. Le and Fu (1996)[35] showed that the $CS_2$ induce chromosome aberration in human sperm. Numerous epidemiological reports concluded that the $CS_2$ is toxicant to viscose industry workers[36-38].

In this study, experimental subjects with smoking habits showed maximum levels of DNA damage when compared to respective controls, which shows that the $CS_2$ exposure with cigarette smoking has synergistic effect on inducing DNA damage. Chromosomal aberrations were shown to be good indicators of future risk of cancer[39]. Likewise, DNA damages are the ultimate causes of cancer because DNA base changes can be mutagenic[40]. The present findings highlight the importance of investigating the genotoxicity of $CS_2$ on viscose plant workers occupationally exposed to this organic solvent when the smoking habit is associated, since this information provides an increased degree of identification for the positive response.

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