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**Original Article**

# Nuclear anomalies in exfoliated buccal epithelial cells of shoe workers in Khartoum state, Sudan

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## ABSTRACT

Shoe polishes product consists of a complex mixture of chemical compounds. Some of these chemicals are known to be absorbed into the body. In this study 50 shoe workers in Khartoum state and 50 controls were tested for cellular abnormalities in buccal mucosa. The age group of the tested individuals ranged between 14 to 49 years. The results of the study showed high frequency of Micronuclei, Karyolysis and Binucleated cells among shoe workers group. These findings indicate the possible role of shoe cleaner products as a source of abnormal changes in buccal mucosa.

**Key Words:** Shoe polishing product, nuclear anomalies, buccal cells, Genotoxicity

## INTRODUCTION

Shoe polishes is known to contain neurotoxic petroleum products that can be absorbed through skin or inhalation. Hexanes are largely unreactive, and easily evaporated non-polar solvents. Occupational hexane poisoning has occurred with Japanese sandal workers, Italian shoe workers, Taiwan press proofing workers. Chinese workers manufacturing I Phones were reported as having suffered hexane poisoning [1]. Toluene exposure is usually associated with simultaneous exposure to several other chemical and the longer it takes for toluene to be eliminated the more harm it is likely to do. Serious adverse behavioral effects are often associated with toluene abuse related to the deliberate inhalation of solvents. Long term toluene exposure is often associated with effects such as: psycho-organic syndrome, visual evoked potential (VEP) abnormality, toxic polyneuropathy, cerebellar, cognitive, and pyramidal dysfunctions, optic atrophy and brain lesions [3][2]. Butanone, also known as methyl ethyl ketone or MEK, is an organic compound, and is an irritant, causing irritation to the eyes and nose of humans,[6], but serious health effects in animals have been seen only at very high levels. When inhaled, these effects included birth defects in mice, but only at the highest dose tested [4][5]. A study to determine the solvents mainly used in shoe making and their genotoxic effects. Occupational exposure was determined by using monitors 3M. Solvents were assessed by gas chromatography. The incidence of nuclear abnormalities was significantly higher in the exposed group when compared to the control group. A positive relationship between the incidence of micronuclei and the toluene concentration in the environment was found [6]. A study of People employed in the shoe manufacture and repair industries are at an increased risk for cancer. The results suggest that occupational exposure to organic solvents, mainly n-hexane, toluene, MEK may cause cytogenetic damage in buccal cells and that use of exfoliated buccal cells seems to be appropriate to measure exposure to organic solvents [7].

## MATERIALS AND METHODS

***Study Population:*** 50 shoe workers were included in this study in addition to control group consists of 50 healthy individuals with no exposure to any toxicant or any other chemical. Both groups were belong to inclusion criteria (smoking cigrate, tobacco and alcohol abuse). Participants are informed about the study, asked to sign the consent form and complete the questionnaires to obtain necessary information on their life style and personal habits (such as age, tribe, working duration, number of shoe boxes per week etc.).

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This study approved by the Faculty of Medical Lab Sciences, Alneelin University, Khartoum.

***Preparation of Buccal Cell Sampling:*** The shoe workers workers and the control group were advised to wash their mouth thoroughly with water to remove unwanted debris. Sterile wooden depressor was used to obtain cell samples from buccal mucosa from each individual. The collected materials then spread on three clean glass slides. Smears were then immediately fixed in 95% alcohol. After fixation, smears were stained by Papincuolae stain, Feulgen reactionand Acridine orange as described elsewhere [8].

***Papincuolae stain:*** Slides stained in harries HX for 7min then washed in distilled water. Slides treated in 0.5M HCL for 10 sec and washed in distilled water. After that treated in Ammoniated water for 2min and washed in distilled water, then treated in 70% alcohol for 2min, treated in 95% alcohol for 2min, treated in 95% alcohol for 2min else. Slides then stained in Orange G for 2min and treated in 95% alcohol for 2min and treated in 95% alcohol for 2min else. Slides stained in EA50 for 3min and treated in 95% alcohol for 1min. Finally cleared and mounted and observed under microscope.

***Feulgen reaction:*** Slides rinsed in 1M HCL at room temperature for 1min and transferred into 1M HCL at 60 degree at oven for 8 min. Then rinsed in 1M HCL at room temperature for 1min. Slides stained in Shiffe reagent for 45 min and washed well in distilled water. Then stained in 1% Light Green for 2 min and washed in water. Let to air dried, and finally cleared, mounted and observed under microscope.

***Acridine orange:*** Slides stained in Acridine Orange solution for 15min and blot by filter paper. Examined as soon as possible by drop of phosphate buffer saline (PBS) under fluorescence microscope used blue light 550nm.

***Scoring Method:*** From each sample three slides were scored, and nuclear abnormalities were classified according to the Tolbert et.al. (1992). these criteria are intended to classify buccal cells into categories that distinguish between "Normal and Abnormal" based on their aberrant nuclear morphology. The abnormal morphologies are due to the DNA damage and cell death. Micronuclei are identified by the presence of main nucleus and one or more smaller nuclei (micronuclei) in cells. The micronuclei are usually round or oval in shape and their diameter may range between 1/3 to 1/16 the diameter of main nucleus. Binucleated cells have two nuclei that are adherent to each other. This is indicative of failed cytokinesis. Karyohixes cells have dense network of nucleochromatin elements that lead to fragmentation and disintegration of the nucleus. In Karyolitic cells, the nucleus is devoid of DNA and appears to have no nuclei. This indicates a very late stage of cell death process.

***Statistical Analysis:*** To determine the frequency of various cell types, 1000 cells were scored for the presence of micronuclei cell, binucleated cells, Karyohetic and Karyolytic cells. All data were expressed as the Mean. The synergistic effect between shoe worker and control group were tested with two way analysis of variance. Multiple comparisons were done by using a least significant difference test. The error rate was accepted as 0.05 values.

## RESULT

**Demographical results:** Table 1 show the main characteristics in subject studied. The mean age group of the selected workers belongs to the range from 13 to 45 years in control group and from 14 to 49 years in the exposed group. They belonged to the similar social economic status. The characteristics of the subject group are mentioned in Table 1(A, B,C and D) . The most age of shoe workers in Khartoum state belong to the range 14 to 24. The most tribe of subject were the Fur then Tama and other tribes. The most of years' experience of shoe workers was belong to the range from one to three years. The main number of product used in polishing by most shoe workers were from one to three boxes per week.

**Cytological results:** Table 2 show the cytological observations. The frequency of micronucleate cell, binucleate cell, karryorhexis cell and karryolysis cell were compared between shoe workers and control group, the mean value of micronuclei in shoe workers was 5.98 against 2.58 in control group in both Papincoulae and Feulgen reaction stain. The mean value of micronuclei in shoe workers was 7.7 against 3.1 in control group in Acridin orange stain. The mean value of binucleate cell in shoe workers was 5.32 against 0.00 in control group in both Papincoulae and Feulgen reaction stain. The mean value of binucleate cell in shoe workers was 5.32 against 0.14 in control group in Acridin orange stain. The mean value of karyohexis cell in shoe workers was 0.92 against 0.00 in control group in both Papincoulae and Feulgen reaction stain. The mean value of karyohexis cell in shoe workers was 1.36 against 0.00 in control group in Acridin orange stain. The mean value of karyolysis cell in shoe workers was 3.92 against 0.00 in control group in both Papincoulae and Feulgen reaction stain. The mean value of karyolysis cell in shoe workers was 4.52 against 0.00 in control group in Acridin orange

stain.Among the four nuclear anomalies, Micronuclei was predominant in exposed subjects compared to control group followed by binucleate cell, karyolytic cell and finally karyohexis cell.

## DISCUSSION

The micronucleus assay in human exfoliated cells is one of the most sensitive methods used for measuring DNA damage rates in human populations; because it is relatively easier to score micronucleus compared to other methods, such as chromosome aberrations. This assay can be used to identify not only groups that are at risk for developing cancer, but also specific individuals who are susceptible to cancer development. Our results make it clear that exposed workers showed an increased frequency of cells with micronuclei, due to the genotoxic effect of the chemical to which they are exposed. Extensive studies and standardized tests to evaluate biological damage at different levels are recommended to public agencies concerned with environmental quality and public health. Mutagenic investigation is one of the necessary evaluations to be done, to ensure environmental quality and occupational health, as is the worker's education about decreasing genetic damage and risk for serious diseases. We advise that charcoal are good alternative cleaner when mixed with solvent such as water, lemon juice to get same result of clearance and shine.

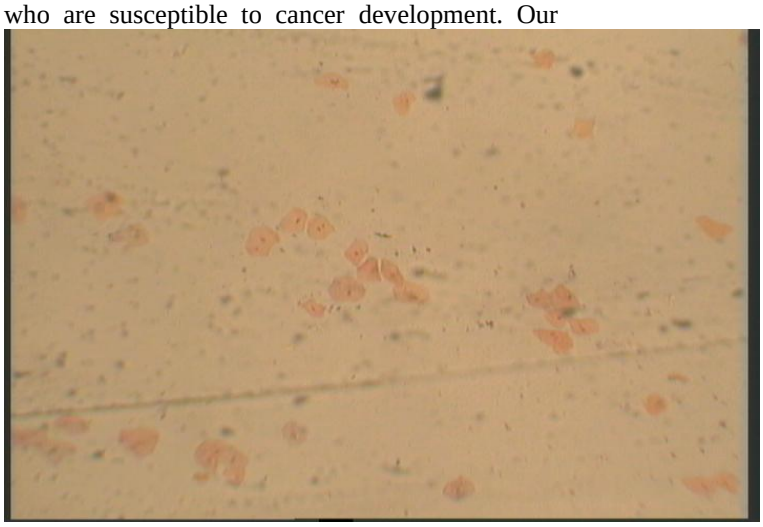

Figure:1 micronuclei and binucleated cell in Papincoulae stain.

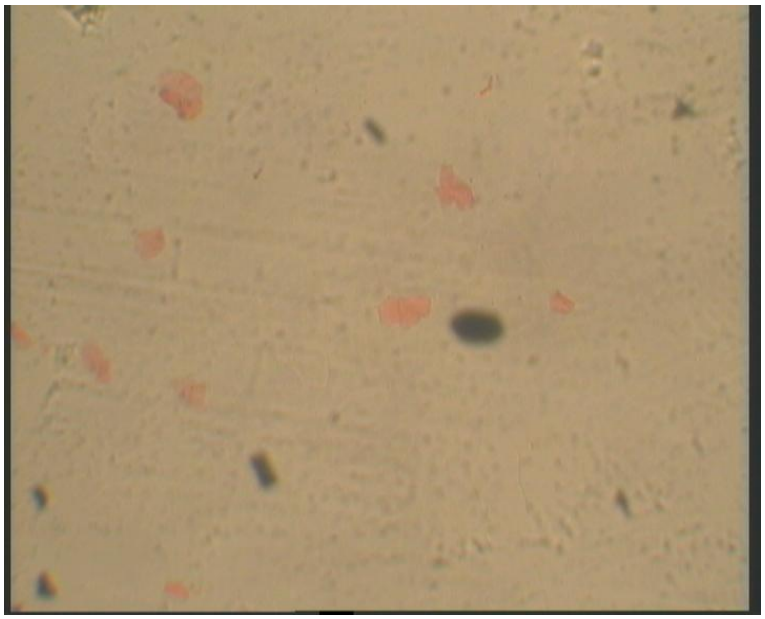

Figure:2 Karyolysis cell in Papincoulae stain.

**Figure:3-A Bincleated cell in Acridin orange stain.**

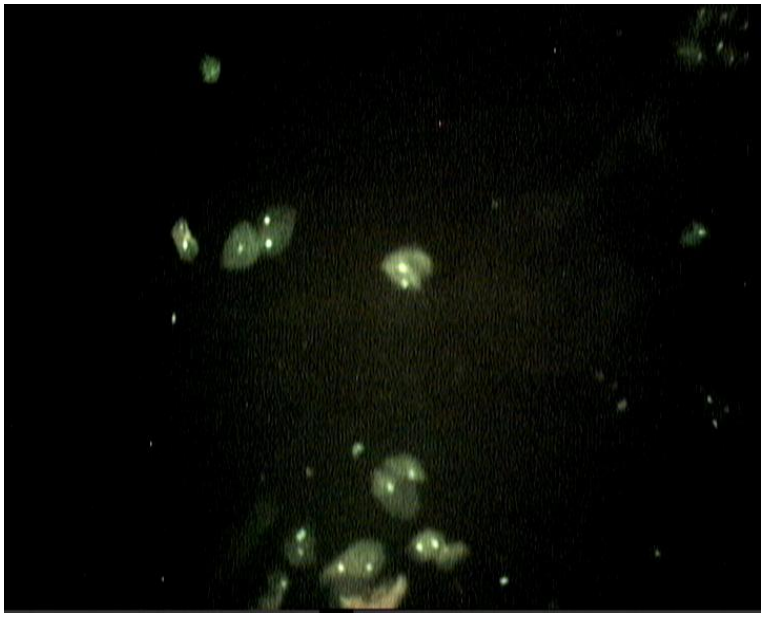

**Figure:3-B Micronuclei and Bincleated cell in Acridin orange stain.**

**Table – 1-A:** ***Demographic Characteristics of Subject.***

| Age | Frequency | Percent |
|---|---|---|
| 14-19 | 15 | 30.0 |
| 20-24 | 15 | 30.0 |
| 25-29 | 8 | 16.0 |
| 30-34 | 8 | 16.0 |
| 35-39 | 2 | 4.0 |
| 40-44 | 1 | 2.0 |
| 45-49 | 1 | 2.0 |
| Total | 50 | %100 |

**Table – 1-B:** ***Demographic Characteristics of Subject.***

| Tribe | Frequency | Percent |
|---|---|---|
| **ashraf** | 1 | 2.0 |
| **brgaoi** | 1 | 2.0 |
| **brgd** | 1 | 2.0 |
| **brnaoi** | 1 | 2.0 |
| **brtaoi** | 2 | 4.0 |
| **dar hamid** | 1 | 2.0 |
| **flati** | 5 | 10.0 |
| **foraoi** | 12 | 24.0 |
| **grnaoi** | 2 | 4.0 |
| **hosaoi** | 4 | 8.0 |
| **kahli** | 1 | 2.0 |
| **mrati** | 3 | 6.0 |
| **mseri** | 1 | 2.0 |
| **tama** | 8 | 16.0 |
| **zgaoi** | 7 | 14.0 |
| **Total** | 50 | %100 |

**Table – 1-C:** ***Demographic Characteristics of Subject.***

| Experience | Frequency | Percent |
|---|---|---|
| less than year | 6 | 12.0 |
| 1-3 | 28 | 56.0 |
| 4-6 | 9 | 18.0 |
| 7-9 | 6 | 12.0 |
| 10-12 | 1 | 2.0 |
| Total | 50 | %100 |

**Table – 1-D:** ***Demographic Characteristics of Subject.***

| No of boxes | Frequency | Percent |
|---|---|---|
| 1-3 | 31 | 62.0 |
| 4-6 | 15 | 30.0 |
| 7-9 | 4 | 8.0 |
| Total | 50 | 100.0 |

**Table – 2***Cytological observation in case control study*

| Cytological observation | | PAP BNC | PAP MNC | PAP KRC | PAP KLC | Feulgen BNC | Feulgen MNC | Feulgen KRC | Feulgen KLC | Acridin BNC | Acridin MNC | Acridin KRC | Acridin KLC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample size | | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| patient | Mean | 5.32 | 5.98 | .92 | 3.92 | 5.3200 | 5.9800 | .9200 | 3.9200 | 5.3200 | 7.7000 | 1.3600 | 4.5200 |
| control | | .0000 | 2.5800 | .0000 | .0000 | .0000 | 2.5800 | .0000 | .0000 | .1400 | 3.1000 | .0000 | .0000 |
| patient | Std. Error of Mean | .593 | .717 | .335 | 1.39 | .5927 | .7174 | .3354 | 1.392 | .5927 | .8232 | .4846 | 1.5634 |
| control | | .00 | .565 | .000 | .000 | .000 | .565 | .000 | .000 | .140 | .677 | .000 | .000 |
| patient | Std. Deviation | 4.192 | 5.07 | 2.3 | 9.8 | 4.191 | 5.072 | 2.37 | 9.845 | 4.191 | 5.821 | 3.427 | 11.05 |
| control | | .000 | 3.995 | .000 | .000 | .000 | 3.995 | .000 | .000 | .989 | 4.790 | .000 | .000 |

***Data are reported as Mean ± SD. *P Value < 0.05 significant level.***

**References**

1-Rizzuto et al. N-hexane polyneuropathy. An occupational disease of shoemakers. European neurology E (1980). 19 (5): 308–15.

2- Urban et al. Neurological and neurophysiological follow-up on workers with severe chronic exposure to toluene. Neurotoxicity . (2003) P25 (s130).

3-Miyagi et al.Tremor induced by toluene misuse successfully treated by a Vim thalamotomy. *Neurology Neurosurgery and Psychiatry* (June 1999) **66** (6): 794–6.

4- Turner et al. The Chemistry of Fire and Hazardous Materials. Boston, Massachusetts: Allyn and Bacon, (1981) Inc. p. 118.

5- Ashford's Dictionary of Industrial Chemicals, 3rd ed, 2011; p 6013-4

6- González-Yebra AL1 et al. Exposure to organic solvents and cytogenetic damage in exfoliated cells of the buccal mucosa from shoe workers.J Occup Med Toxicol. 2010 Aug 20;5(1):25.

7-Burgaz S1 et al. Cytogenetic analysis of buccal cells from shoe-workers and pathology and anatomy laboratory workers exposed to n-hexane, toluene, methyl ethyl ketone and formaldehyde ,Biomarkers. 2006 Jul-Aug;11(4):383.

8-John D .Bancroft, Marilyn Gamble. Theory and Practice of Histological Techniques. 5th ed, 2002; pp 237-631.