

## Nuclear Anomalies in Exfoliated Buccal Epithelial Cells of Shoe Workers in Khartoum State

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### Abstract

Shoe polishes product consists of a complex mixture of chemical compounds. Some of these chemical 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. For this a questionnaire based survey was conducted. Simultaneously, buccal smears were collected from oral cavity of the selected individuals and tested for nuclear anomalies. 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.

**Keywords:** Shoe polishing product; Nuclear anomalies; Buccal cells; Genotoxicity

### Introduction

Shoe polishes contains neurotoxic petroleum products that can be absorbed through skin or inhalation. The study was done in the Khartoum state and tested for cellular abnormalities in buccal cavities by screening several methods. 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 [2,3]. 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 [4], 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 [5] A study to determine the solvents mainly used in shoe making and their genotoxic effects. Occupational exposure was determined by using monitors 3 M. 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 [4]. A study of People employed in the shoe manufacture and repair industry 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 [6].

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

#### Preparation of buccal cell sampling

The shoe 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 of buccal mucosa from each individual. on three clean glass slides Smears were seen and then immediately they were fixed in 95% alcohol. After fixation, smears were stained by Papincuolae stain, Feulgen reaction and Acridine orange as described elsewhere [7].

#### Papincuolae stain

Slides stained in harries HX for 7 min then washed in distilled water. Slides treated in 0.5 M HCL for 10 sec and washed in distilled water. After that treated in Ammoniated water for 2 min and washed in distilled water, then treated in 70% alcohol for 2 min, treated in 95% alcohol for 2 min, treated in 95% alcohol for 2 min else. Slides then stained in Orange G for 2 min and treated in 95% alcohol for 2 min and treated in 95% alcohol for 2 min else. Slides stained in EA50 for 3 min and treated in 95% alcohol for 1 min. Finally cleared and mounted and observed under microscope [8].

#### Feulgen reaction

Slides rinsed in 1 M HCL at room temperature for 1 min and

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transferred into 1 M HCL at 60 degree at oven for 8 min. Then rinsed in 1 M HCL at room temperature for 1 min. Slides stained in Shiffre 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 15 min and blot by filter paper. Examined as soon as possible by drop of phosphate buffer saline (PBS) under fluorescence microscope used blue light 550 nm.

### Scoring method

From each sample three slides were scored, and nuclear abnormalities were classified according to the Tolbert et al. 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 Karyolytic 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, Karyorrhetic 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	Frequency	Percent
14-19	15	30
20-24	15	30
25-29	8	16
30-34	8	16
35-39	2	4
40-44	1	2
45-49	1	2
Total	50	100%

Table 1A: Demographic characteristics of subject.

Demographic Characteristics of Subject.		
Tribe	Frequency	Percent
Ashraf	1	2
Brgaioi	1	2
Brgd	1	2
Brnaoi	1	2
Brtaoi	2	4
Dar hamid	1	2

Flati	5	10
Foraoi	12	24
Grnaoi	2	4
Hosaoi	4	8
Kahli	1	2
Mrati	3	6
Mseri	1	2
Tama	8	16
Zgaoi	7	14
Total	50	100%

Table 1B: Demographic characteristics of subject.

Experience	Frequency	Percent
Less than year	6	12
3-Jan	28	56
6-Apr	9	18
9-Jul	6	12
12-Oct	1	2
Total	50	100%

Table 1C: Demographic characteristics of subject.

No of boxes	Frequency	Percent
3-Jan	31	62
6-Apr	15	30
9-Jul	4	8
Total	50	100

Table 1D: Demographic characteristics of subject.

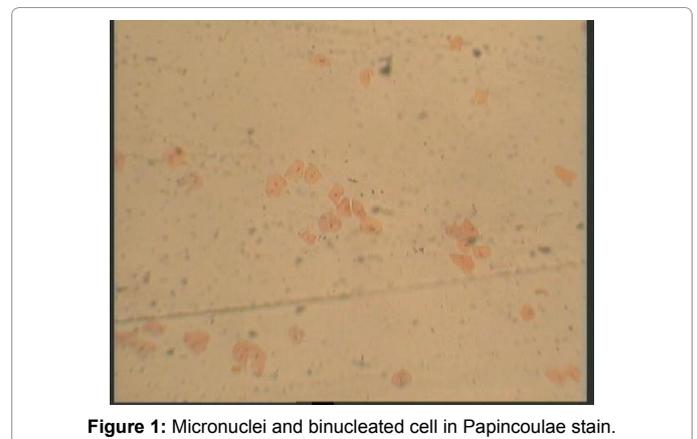


Figure 1: Micronuclei and binucleated cell in Papincolae stain.

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 Tables 1A-1D. 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 year's 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 (Figure 1-3).

### Cytological results

Table 2 show the cytological observations. The mean value of micronuclei in shoe workers was 5.98 against 2.58 in control group in both Papincolae 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

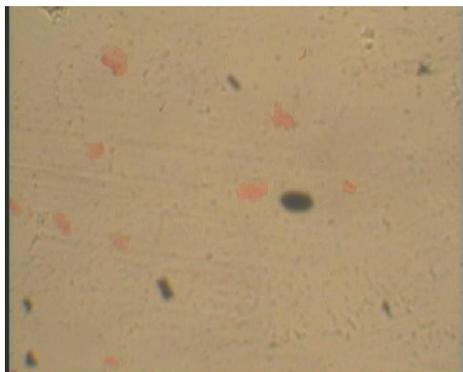


Figure 2: Karyolysis cell in Papincolae stain.



Figure 3A: Binucleated cell in Acridin orange stain.

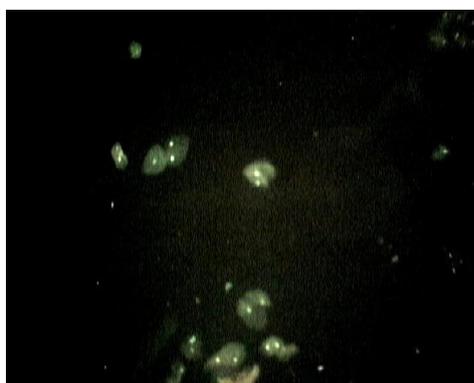


Figure 3B: Micronuclei and Binucleated cell in Acridin orange stain.

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	0.92	3.92	5.32	5.98	9200	3.92	5.32	7.7	1.36	4.52
Control		0	2.58	0	0	0	2.58	0	0	1400	3.1	0	0
Patient	Std. Error of Mean	0.593	0.717	335	1.39	5927	7174	0.3354	1.392	5927	8232	4846	1.5634
Control		0	565	0	0	0	0.565	0	0	140	677	0	0
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		0	3.995	0	0	0	3.995	0	0	0.989	4.79	0	0

Table 2: Cytological observation in case control study. Data are reported as Mean  $\pm$  SD. \*P Value <0.05 significant level.

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. Micronuclei exposed subjects compared to control group followed by binucleate cell, karyolytic cell and finally karyohexis cell (Table 2).

## 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 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 advice that charcoal are good alternative cleaner when mixed with solvent such as water, lemon juice to get same result of clearance and shine.

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