Evaluation of Genotoxic Effects of Formaldehyde in Adult Albino Rats and Its Implication In Case of Human Exposure

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Abstract: Background: Formaldehyde is a reactive chemical that is commonly used in the production of industrial, laboratory, household, and cosmetic products. Formaldehyde (FA) is a potential carcinogen and mutagen. **Objectives:** This study was designed to evaluate the systemic genotoxicity of formaldehyde in experimental animals and in subjects exposed to FA. **Material, Subjects and Methods**: The animal study included twenty one rats that were divided into Group (1): Negative control rats ,Group (2): Positive control rats: received daily intraperitoneal injected with distilled water ,Group (3): Formaldehyde group, received single intraperitoneal injection of Formaldehyde (0.2 mg/kg/day) after 4 weeks of treatment, rats were sacrificed then submitted to cytogenetic examination by detection of their chromosomal pattern and mitotic index in bone marrow cells. The human study comprised two groups: 30 individuals occupationally exposed to formaldehyde in Zagazig University (cases) and 15 unexposed individuals (controls), from whom peripheral blood were collected and used for evaluation of the chromosomal aberrations (CAs) frequency and the comet assay for detection of DNA damage.**Results:** This study revealed increased frequency of structural chromosomal aberrations and decreased mitotic index of bone marrow cells of rats exposed to FA. Individuals exposed to FA also showed high frequency of chromosomal aberrations and increased levels of DNA damage in the Comet assay in terms of tail length and tail moment in peripheral blood lymphocytes compared to controls. **Conclusion:** Exposure to formaldehyde induced Chromosomal aberrations and DNA damage in peripheral blood lymphocytes of exposed subjects and bone marrow cells of albino rats.
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1. Introduction

Formaldehyde (FA), a member of the aldehyde family, is a bactericidal agent and tissue preservative **(Naya and Nakanishi, 2005; Yamato *et al.*, 2005).** It is found in nature, domestic air, cigarette smoke, and the polluted atmosphere of cities **(Songur *et al.*, 2003).** It is widely used in industrial and medical settings. Employees, especially the histologist, anatomist, pathologist and medical students following dissection lectures, are the subjects most frequently exposed to FA gas. FA in excess of certain doses is accepted as being toxic, and its harmful effects increase under room temperature due to easy evaporation and also metabolism into formic acid **(Gurel *et al.*, 2005 and Yamato *et al.*, 2005).**

Physiological FA can be formed by the metabolism of L-methionine, histamine or methylamine, and can contribute to biological methylation by folic acid **(Trezl *et al.*, 1990).**

Formaldehyde (FA), being a very reactive compound, can react with different macromolecules, such as proteins and nucleic acids or with low molecular weight substances as amino acids **(Cheng *et al.*, 2003 ; Metz *et al.*, 2004).** The inhalation of FA gas can produce irritation to eyes, nose and the upper respiratory tract. Whilst occupational exposure to high FA concentrations may result in respiratory irritation and asthmatic reactions. It may also aggravate a pre-existing asthma condition. Skin reactions following exposure to FA are very common, because the chemical is both irritating and allergenic **(Pala *et al.*, 2008).** FA induces genotoxic and cytotoxic effects in bacteria and mammalian cells **(Usanmaz *et al.*, 2002)** and its carcinogenicity have been proven in experimental studies that used proliferating cultured mammalian cells and human lymphocytes. Furthermore, FA can act as a cell-proliferation retardation factor and mediates the apoptotic process **(Speit *et al.*, 2007 and Pala *et al.*, 2008).**

Tyihak *et al.*, 2001and Marcsek *et al.*, 2007 concluded that formaldehyde can act as a cell-proliferation retardation factor, and can mediate the apoptotic process. In cell cultures, **Zhang *et al.*, 2009** found that formaldehyde at concentrations around 10mM decreased apoptosis and increased cell proliferation, whereas higher doses enhanced apoptosis and decreased cell proliferation**.** At the molecular level, FA exposure may generate reactive

oxygen species (ROS) inducing the formation of DNA molecular adducts **(Bono *et al.*, 2010).**
The aim of this study was to evaluate the systemic genotoxicity of formaldehyde in experimental animals and in subjects exposed to formaldehyde.

2. Material, Subjects and Methods:

Study design and setting

The current work was composed of 2 studies. The 1st one was the experimental study which carried on 21 adult albino rats and the 2nd one was the human study which carried on 45 subjects.

I. Experimental Study:

Twenty one adult male albino rats weighing 150-200 gm were obtained from the Animal House of the Faculty of Medicine, Zagazig University. The animals were divided into 3 groups:
Group (I): Negative control rats
Group (II):Positive control rats: received daily intraperitoneal injected with distilled water (0.5 ml once/day).
Group (III): Formaldehyde group, received single intraperitoneal injection of Formaldehyde (0.2 mg/kg/day) (1/10 LD_{50}) **(Odeigah, 1997)** for 4 weeks. Twenty four hours after the last injection, the rats were sacrificed then submitted to cytogenetic examination by detection of their chromosomal pattern and mitotic index in bone marrow cells.

Cytogenetic study

Cytogenetic study was performed using a known bone marrow technique described by **Speit *et al.* (1992).** Animals were injected I.M., 2 hours before scarification with 0.25ml/ 100g B.W. 0.5% colchicines. Both femora were dissected out and cleaned of any adhering muscle. Bone-marrow cells were collected from both femora by flushing potassium chloride (KCl) (0.075M, at 37ºC), and incubated at 37ºC for 25 min. Collected cells were centrifuged at 2000 × g for 10 min, and fixed in aceto-methanol (acetic acid: methanol, 1:3, v/v). Centrifugation and fixation were repeated five times at an interval of 20min. The cells were resuspended in a small volume of fixative, dropped onto chilled slides, and allowed to dry. They were stained the following day with freshly prepared 2% Giemsa stain for 3–5 min, and washed in distilled water to remove excess stain, For each animal 100 well prepared). Metaphases were analyzed and different types of chromosomal abnormalities were recorded.

Mitotic Index determination.

The mitotic index was used in order to determine the rate of cell division. In order to calculate the mitotic index the slides that were prepared for the chromosomal aberration assay were used. Random views on the slides were selected to determine the number of dividing cells (metaphase stage). At least 1000 cells were examined in each preparation. The mitotic indices were obtained by counting the number of mitotic cells in 1000 cells per animal to a total of 5000 cells per treatment and control under an Olympus microscope. The mitotic index was calculated as the ratio of the number of dividing cells to the total number of cells, multiplied by 100 **(Shukla *et al.*, 2002).**

II. Human Study:

The study population consisted of 30 Lab technicians and workers occupationally exposed to formaldehyde in Pathology, Histology and Anatomy laboratories at Zagazig University. A control group of 15 non-exposed subjects was considered. All subjects were informed about the nature of the study and written informed consents were obtained from all studied subjects. The protocol of the study was approved by the Ethical Committee for Research, Faculty of Medicine, Zagazig University. Medical history, medication and lifestyle factors for all studied individuals, as well as information related to working practices (such as years of employment) were obtained. The characteristics of both groups are described in Table (1).

Venous Blood samples (5ml) were collected into sodium-heparin tubes. Immediately after sampling the blood was put on ice and brought to the laboratory for analysis. The same blood sample was analyzed using both methods: chromosomal aberration analysis and the Comet assay.

Genotoxicity assessment:

I- Analysis of chromosomal aberrations:

Analysis of chromosomal aberrations was used to detect cytogenetic damage according to the method of **Verma (1998).** Cultures were established by adding the blood sample to a culture medium containing Roswell Park Memorial Institute (RPMI) 1640 medium (pH 6.8–7.2), 20% fetal calf serum, 6 μg/ml phytohemagglutinin L (PHA-L), 0.5 mg/ml L-glutamine, and antibiotics (100 IU/ml penicillin, 100 μg/ml streptomycin) and incubating for 72 h at 37°C. Colcemid was added 2 h before harvesting, to stop mitosis at metaphase and prevent spindle formation. After hypotonic treatment with potassium Chloride (KCl), the cells were fixed in methanol/acetic acid (3:1), spread on wet slides and chromosome slides were stained with 5% Giemsa in phosphate buffer (pH 6.8) for 10 min. On average, 50-100 metaphases were analyzed from each individual using the high power oil immersion length for the presence of chromosomal aberration.

II- Comet assay

The assay was conducted under alkaline conditions according to **Singh *et al.* (1988).** Blood samples were suspended in 0.5% low melting point agarose and sandwiched between a layer of 0.6%

normal melting point agarose and a top layer of 0.5% low melting point agarose on fully frosted slides. During polymerization of each gel layer the slides were kept on ice. After solidification of the 0.6% agarose layer the slides were immersed in lysis solution (1% sodium sarcosinate, 2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris–HCl, 1% Triton X-100 and 10% Dimethyl sulfoxide (DMSO) at 4°C. After 1 h slides were placed in electrophoresis buffer (0.3 M NaOH, 1 mM Na_2EDTA, pH 10) for 20 min at room temperature to allow for DNA unwinding. Electrophoresis was conducted in a horizontal electrophoresis platform in fresh, chilled electrophoresis buffer for 20 min at 300 mA and 19 V. The slides were neutralized with Tris–HCl buffer, pH 7.5, three times for 5 min and stained with 10% ethidium bromide for 10 min. Each slide was analyzed using a fluorescence microscope equipped with a 515–560 nm excitation filter. For each subject 50 cells were analyzed with an automatic digital analysis system, Comet assay II (Perceptive Instruments, Halstead, UK), determining tail length and tail moment (tail length×% tail DNA/100).

Statistical analysis*:*

SPSS Software program was used. Quantitative data were compared using student's t test, while qualitative data were compared using chi square test. Correlation coefficient (r) was used for testing the association between two continuous variables. The significance level is considered at *P* value < 0.05.

Table 1: demographic characteristics of the studied population(n 45) grouped according to exposure status.

	Number of subjects	Gender		Age		Years of employment	
		Males	Females	mean±SD	Range	mean±SD	Range
Exposed group	30	20	10	42.5±6.3	35-50	14.3±2.5	10-20
Control group	15	8	7	39.3±5.6	34-53	-	-

3. Results:

I. Experimental Study:

Regarding the control groups (group I and II), there was no statistically significant difference between these two groups in the frequency of chromosomal anomalies or mitotic index (Table 2 and Figs. 1,2).

The rats exposed to formaldehyde through intra peritoneal injections (group III) showed a significant increase in the total abnormal metaphases which represented by various chromosomal anomalies in the form of chromatid gap and break, chromatid fragment, chromatid deletion, Ring chromosome, and Dicentric chromosome. All these anomalies showed a significant increased frequency as compared to the corresponding control group (Table 2 and Fig. 1).

Numerical anomalies including aneuploidy and polyploidy also noticed in this group. These numerical anomalies showed a non significant change, compared to the control group. Also mitotic index showed a significant decrease in Formaldehyde exposed rats as compared to the control values Fig. (2).

Table 2 : Statistical analysis of the chromosomal aberrations in bone marrow cells of the control rats and Formaldehyde treated rats, after 4 weeks of the study.

Chromosomal Anomalies	**Group 1 (-ve Control) n= 7 (Mean ± SE)**	**Group II (+ve Control) n= 7 (Mean ± SE)**	**Group III Formaldehyde group n= 7 (Mean ± SE)**
I- Structural anomalies			
- Chromatid gap and break	9.4±0.12	8.2±0.17	17.1±0.05*
- Chromatid deletion	8.5±0.13	7.7±0.09	15.6±0.08*
- Ring chromosome	6.3±0.08	7.1±0.10	12.6±0.09*
- Dicentric chromosome	3.2±0.03	2.9±0.09	9.0±0.11*
- Stretched chromosome	6.2±0.09	5.9±0.11	10.5±0.08*
-Total abnormal metaphase	33.6±0.12	31.8±0.12	54.8±0.5*
II- Numerical anomalies			
- Aneuploidy	00.0±0.0	00.0±0.0	1.5±0.03
- Polyploidy	0.5 ±0.02	0.3 ±0.05	3.5±0.04

Data are expressed as mean value chromosomal aberrations /100 metaphases ± SE

* Significantly different from the controls (Student's t-test; $p < 0.05$)

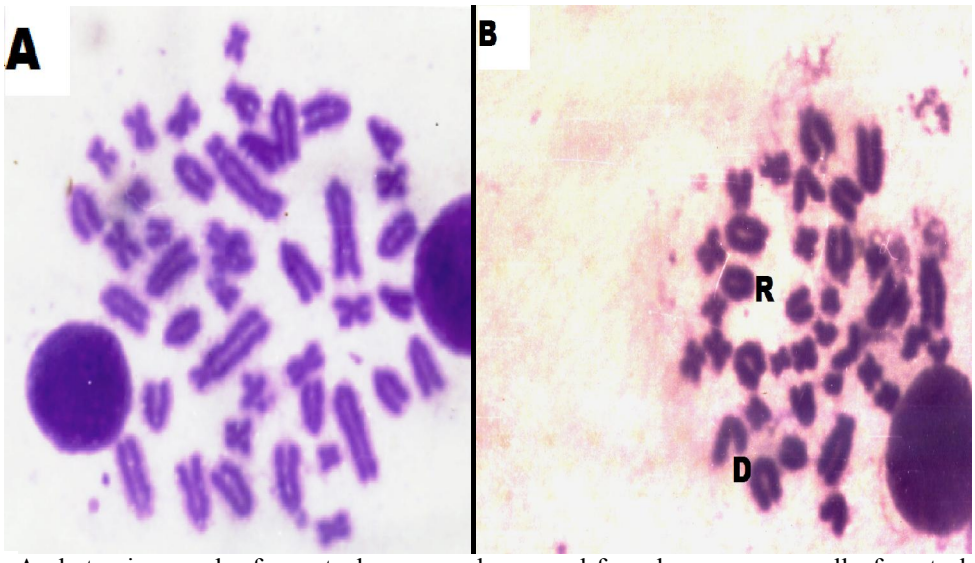


Fig 1: A photomicrograph of a metaphase spread prepared from bone marrow cell of control group (A) showing normal chromosomal structure and number and formaldehyde treated group (B) showing more than one type of chromosomal aberrations as deletion [D] and ring chromosome [R]) (Giemsa stain x 1000).

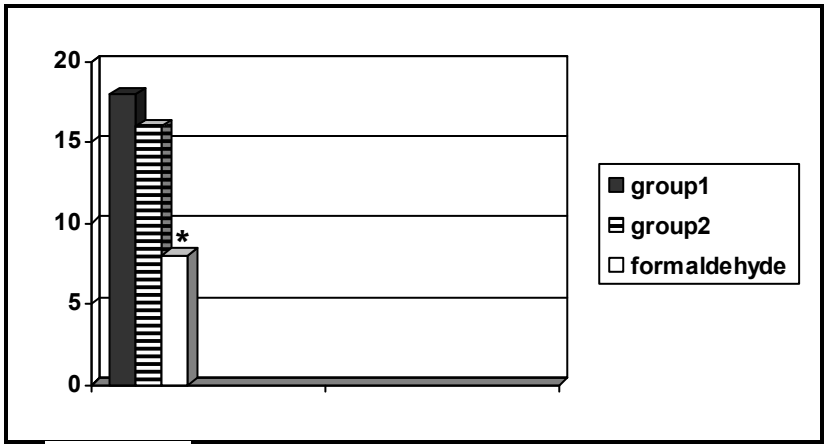


Fig 2: Bar chart shows the Mitotic index of bone marrow cells in control groups and formaldehyde treated rats (* significantly different from the control, Student's t-test, $p < 0.05$).

II. Human Study

The results of structural chromosomal aberrations analysis in human peripheral blood lymphocytes were shown in Tables 3 and 4. It was observed that formaldehyde exposure induced a statistically significant increased number of aberrant cells with chromatid gap and break, chromatid deletion, ring chromosome and dicentric chromosome as compared to the corresponding values in the control group. While numerical anomalies including aneuploidy and polyploidy showed a non significant change compared to the control group. A multivariate analysis of variance showed that gender and age did not affect the type of structural aberrations. Also, non significant differences in the numbers of aberrations with regard to subject gender were found Table (4).

The effects of Formaldehyde exposure on the extent of DNA migration (Comet assay) were presented in Tables 5, 6 Fig. 3. The exposed group peripheral blood lymphocytes showed significantly increased levels of DNA damage in terms of tail length and tail moment as compared to the corresponding values in the control group. Distribution of both Comet assay end-points appeared to be wider in Formaldehyde exposed subjects, compared to the controls. Point values of tail lengths ranged from 16 to 74 µm, whereas the values of tail moments ranged from 11 to 88 µm. The values of tail lengths distribution for the control subjects were from 7 to 14 µm and tail moments from 5 to 13. The multivariate analysis of variance showed that the gender and the age did not affect between-group variations in the Comet assay parameters Tables (6).

Table 3: Statistical analysis of chromosomal aberrations in peripheral blood lymphocytes of the control group and the Formaldehyde exposed group.

Chromosomal aberrations	Control group n= 15 (Mean ± SE)	Formaldehyde Exposed group n= 30 (Mean ± SE)
I- Structural		
- Chromatid gap & break	1.9±0.36	6.5± 0.65*
- Chromatid deletion	8.7±0.55	15.5±0.47*
- Ring chromosome	5.5±0.33	16.4±0.29*
- Dicentric chromosome	0.9±0.41	9.0±0.54*
-Total abnormal metaphase	20.0±0.27	46.4±0.35*
II- Numerical		
- Aneuploidy	0.2±0.12	0.7±0.10
- Polyploidy	0.6±0.14	0.9 ±0.09

Data are expressed as mean value chromosomal aberrations /100 metaphases ± SE
* Significantly different from the control group (Student's t-test; $p < 0.05$).

Table 4: Statistical analysis of chromosomal aberrations in peripheral blood lymphocytes of the control and the Formaldehyde exposed groups according to the gender of the subjects.

Chromosomal aberrations	Control group (Mean ± SE)		Formaldehyde Exposed group (n= 30) (Mean ± SE)	
	Male n= 8	Female n= 7	Male n= 20	Female n= 10
I- Structural				
- Chromatid gap & break	1.4±0.22	1.6±0.21	7.1± 0.44	6.4± 0.49
- Chromatid deletion	8.1±0.38	7.7±0.32	16.5±0.19	15.9±0.23
- Ring chromosome	5.0±0.37	5.8±0.32	16.9±0.38	15.6±0.32
- Dicentric chromosome	0.9±0.40	0.8±0.37	9.0±0.51	8.0±0.49
-Total abnormal metaphase	15.4±0.20	15.1±0.18	48.5±0.36	44.9±0.29
II- Numerical				
- Aneuploidy	0.2±0.11	0.2±0.13	0.7±0.2	0.8±0.10
- Polyploidy	0.6±0.09	0.7±0.14	0.9 ±0.05	0.7 ±0.09

Table 5: Statistical analysis of Comet assay end-points in peripheral blood lymphocytes of the control and the Formaldehyde exposed groups.

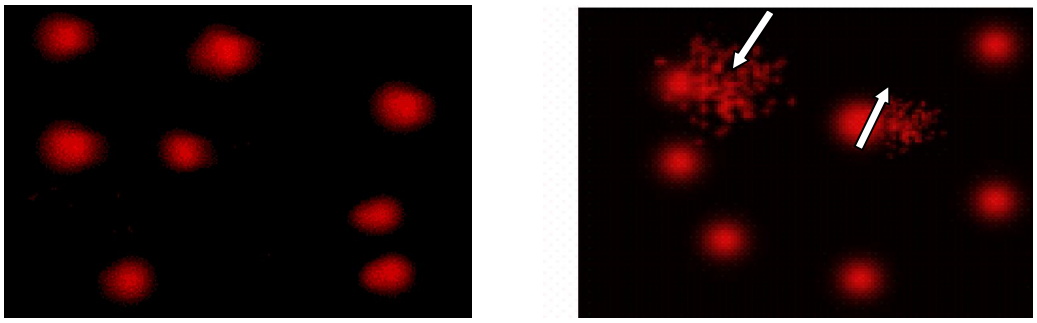
Groups	Tail length (µm)	Tail moment	Tail length distribution (µm)	Tail moment distribution
Control group	12.5±1.5	10.8±1.2	7.2-14.7	5.8-13.6
Formaldehyde Exposed group	47.3±8.5*	56.1±16.5*	16.5-74.2	11.4-88.1

Data are expressed as mean value of end-points /50 comets ±SE
* Significantly different from the control group (Student's t-test; $p < 0.05$)

Table 6: Comet assay end-points in peripheral blood lymphocytes of the control and the Formaldehyde exposed groups according to the gender of the subjects.

Groups	Tail length (µm)	Tail moment
Control group		
- Male	12.2±1.8	10.5±1.2
- Female	13.1±1.2	11.7±0.9
Exposed group		
- Male	45.9±8.8	59.2±7.85
- Female	46.1±9.1	57.4±15.2

Data are expressed as mean value of end-points /50 comets ±SE



A B

Fig 3: Comet assay in peripheral blood lymphocytes of the control group (A), showing no DNA damage and the Formaldehyde exposed group (B), showing DNA damage (⟶).

4. Discussion

A relevant indoor exposure to Formaldehyde (FA) is detectable in hospitals and scientific institutions where FA is used as a bactericide and tissue preservative **(Kurose *et al.*, 2004).** Histology, pathology, cadaver embalming technicians, dissection students and nurses working at dialysis units are occupationally subjected to FA exposure **(Kilburn *et al.*, 1987).**

Nevertheless, the Formaldehyde genotoxic potential in occupationally exposed individuals is conflicting. Chromosomal damage is considered to detect early effects of xenobiotic insult and evaluation of the frequency of chromosomal aberrations, as being a sensitive cytogenetic assay for detecting exposure to mutagens and carcinogens **(Bonassi *et al.*, 1995).**

The results of this study demonstrated that Formaldehyde injections in rats induced a significant increase in the total abnormal metaphases which showed various chromosomal anomalies in the form of chromatid gap and break, chromatid fragment, deletion, ring chromosome and dicentric chromosome which showed a significantly increased frequency as compared to the control group. Also mitotic index showed a significant decrease in Formaldehyde exposed rats as compared to the control group values.

These results coincided with those of **IARC, (2006)** who reported that long-term exposures to high concentrations of Formaldehyde appear to have a potential for inducing DNA damage. These effects were well demonstrated in experimental studies with animals, in which local genotoxic effects following FA exposure were observed, i.e. DNA–protein cross-links and chromosome damage.

Also **Cao *et al.*(2009)** observed in their study a significant increase in the micronucleus rate and chromosome aberration (mainly for chromosome breakage, polyploid) in liver of embryos after maternal exposure to formaldehyde.

The mitotic index is one of the standard indices commonly used for measuring cytotoxicity. A significant decrease in mitotic index was observed in rats treated with formaldehyde. This significant inhibition of mitotic activity of bone marrow cells observed in this study may be correlated with DNA damage or apoptosis induced by formaldehyde **(Tyihak *et al.*, 2001 and Costa *et al.*, 2008).**

On studying the results of the clinical part of present research work, it showed that the peripheral blood lymphocytes of the Formaldehyde exposed subjects had a statistically significant increase in the number of aberrant cells with chromatid gap and break, chromatid deletion, ring and dicentric chromosomes. These results suggest that long-term exposure to Formaldehyde induced a significant increase in the number of both chromatid and chromosomal types of aberrations. This finding is supported by the appearance of dicentric and ring chromosomes as complex aberrations that were not of statistical significance in the control subjects. Formaldehyde induced chromosomal aberrations (CAs) could be attributed to disrepair of lesions in the G_0 stage of circulating lymphocytes as suggested by **Carrano and Natarajan (1988).** The chromosome types of aberrations could also arise due to increase in DNA lesions or interference with their repair **(IAEA, 1986).**

These results agree with those presented by **Schmid *et al.* (1986)** who described a significant increase of the chromatid type CA and sister chromatid exchange (SCE) in human lymphocytes treated with FA in vitro while, **Shaham *et al.* (2002)** reported an increased level of DNA-protein crosslinks and SCE in industry workers exposed to FA. Also **He *et al.* (1998)** observed a significant increase in MN, CA and SCE in PBL of FA-exposed anatomy students.

Jakab *et al.* (2010) demonstrated that exposure to formaldehyde induces apoptosis and

CAs, indicating an excess cancer risk among subjects occupationally exposed to formaldehyde. At this concern, in some biomonitoring studies, cytogenetic effects, such as increased sister chromatide exchanges (SCE), chromosomal aberrations (CAs), and micro nucleated cells (MNC), were described **(Shaham *et al.*, 1997; Burgaz *et al.*, 2001 and Ye *et al.*, 2005),** while in other reports, these evidences were lacking **(Thomson *et al.*, 1984).**

Chromosome damage and effects on lymphocytes arise because FA escapes from sites of direct contact, such as the mouth, causing nuclear alterations in the lymphocytes of those exposed **(He, *et al.*, 1998 ; Orsière *et al.*, 2006 and Zhang *et al.*, 2009).**

Our results thus corroborate previous reports **(Viegas *et al.*, 2010 and Ladeiraa *et al.*, 2011)** that lymphocytes can be damaged by long-term FA exposure. Moreover, the changes in peripheral lymphocytes indicate that the cytogenetic effects triggered by FA can reach tissues far away from the site of initial contact **(Suruda *et al.*, 1993).).** In general the genotoxic effects of FA exposure on first contact tissues such as the nose and the respiratory tract have been increasingly convincing. A relationship between FA exposure and micronuclei (MN) frequencies in both buccal smears and nasal mucosa of workers in anatomy and pathology laboratories was demonstrated by **Burgaz *et al.* (2001)** and by **Costa *et al.* (2008).**

Only in the last few years the Comet assay has being introduced as a useful technique in human biomonitoring studies allowing the evaluation of DNA damage at the single cell level. Therefore, few are the studies published on FA occupational exposure in which this biomarker is used. However, there are already some in vitro studies in cellular lines and in animal and human leukocytes culture cells in which the Comet assay proved to be a sensitive biological indicator in the evaluation of the genotoxic effect of FA **(Frenzilli *et al.*, 2000; Liu *et al.*, 2006 and Sul *et al.*, 2007).**

The current study showed that the levels of DNA damage, measured as tail length (TL), were significantly increased in the Formaldehyde exposed group compared with the control group. This result agrees with those presented by **Yu *et al.*(2005)** who reported a significantly increase of TL and olive tail moment in peripheral blood lymphocyte of 151 workers from two plywood factories. DNA damage detected in the present study and measured as Comet assay end-points could possibly originate from DNA single-strand breaks, repair of DNA double-strand breaks, DNA adduct formation or DNA–DNA and DNA–protein cross links (**King *et al.*, 1993 ; *et al.*, 1995 and Shah *et al.*, 1997).**

5. Conclusion:

The current work demonstrated that exposure to Formaldehyde induced chromosomal aberrations and DNA damage in the peripheral blood lymphocytes of exposed subjects and bone marrow cells of albino rats.

6. Recommendations:

The results presented in this study emphasize the importance of personal protection at work places, with possible occupational exposure to Formaldehyde. The results also stress the necessity of a further more detailed testing of genotoxicity in subjects occupationally exposed to Formaldehyde, in order to detect early cytogenetic biomarkers of exposure and to prevent further induction of DNA lesions which could induce neoplastic growth of damaged somatic cells.

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