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# Study of genotoxic and cytotoxic effects of tobacco and Urethane in lung and colorectal of white mice

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## ABSTRACT
The genotoxic and cytotoxic effects of tobacco and urethane were studied and the results using the cytogenetic endpoints: micronucleus test (MN) in polychromatic erythrocytes (PCEs), chromosome aberration analysis in the bone-marrow of white mice *Mus musculus*. In this observation,, The impact of various doses of cigarette smoke and mouse lung urethane on the lung and the colorectal morphological changes, both in the lung and in the colorectum, is further examined. The cigar mice were then exposed to smoke once or twice a day in a locked environment five times a week. urethane after injection of Interpretation (5mg / kg). The effect of various doses of mouse lung and colorectal tobacco smoke and urethane-induced mouse lung also tests morphological modifications in both the lung and colorectal of the mice. The animals were divided into six groups at random, while the negative (control) group got distal water only. The results of the statistical analysis indicated that micronucleus immature blood cells in the bone marrow had a significant increase of $P \leq 0.05$ for both urethane and tobacco treatment in the formation of micronuclei compared to negative control. The study findings also indicated that the induction of chromosomal aberration of immature red blood cells in the bone marrow significantly increased by $P \leq 0.05$. Also the results showed number Tobacco treatment and urethane induced histological changes in both lung and colorectal tissue of nodules and hyperplasia amongst these various groups, as revealed by light microscope. In conclusion, current study revealed that tobacco and Urethane have high genotoxicity and Cytogenetically effects on somatic cells of white mice, also ever smokers have an increased risk of lung and colon cancer.

## Introduction
Smoking is a process in which a particular substance is burned, and tobacco is the substance most commonly used in it, and a person tastes it and inhales it after it is burned. Many people resort to smoking because they consider it a practice that helps in recreation, and in some religious rituals it is sometimes practiced to impart a state of spiritual, smoking just one hookah and a pipe. Approximately 10 mg of nicotine are included in each cigarette [1].

The need for attention to harms of smoking and the diseases caused by stroke should be made aware of the harms of smoking. Cardiac arrest, Respiratory diseases ,Cancer, especially lung cancer. Smoking cigarettes or tobacco rolls is a serious and certainly bad habit, as a cigarette contains 400 chemical substances, including the most carcinogenic or infertile combination [2] .

Smoking cigarettes causes cancer can be characterized as a disease in which, by following the usual rules of cell division, a group of abnormal cells form and spread uncontrollably. It can lead to death if the spread is not managed. Cancer is caused by external causes, such as inherited genetic mutations, hormones, and immune disorders, such as cigarettes, infectious species, and an unhealthy diet, and internal factors. Together or in sequence, these factors can work to cause cancer. Between exposure to environmental factors and observable cancer, ten or more years often pass. [3] [4].

Inhaled cigarette smoke causes damage of adduct formation to the lungs and nasal mucosa [5]. Tobacco is responsible for about one in three of all cancer deaths in the developed world, tobacco smoking is associated with many forms of cancer (and causes 90% of lung cancer, over fifty known carcinogens, are present tobacco smoke as nitrosamines and polycyclic aromatic hydrocarbons Decades of research show the relation between tobacco use and cancer in the lung[5][6] also some chemical carcinogen (urethane), by several lines of evidence indicate that ethyl carbamate (urethane induced) lung tumor formation, a prototypical mouse model of multistage lung carcinogenesis [7].

Urethane (ethyl carbamate) is a mutagenic, carcinogenic and hepatotoxic anesthetic used to provide anesthesia adequate for surgical procedures lasting up to 8 or more hours [7][8]. Long periods of

urethane use are reported to cause respiratory system depression. The most common cause of cancer death worldwide is lung cancer, which is close to human lung morphology, histology, and molecular characteristics of mouse lung tumors[8]. It has also become clear that the risk of non-smokers developing lung cancer is increased by exposure to ambient tobacco smoke [9]. Adenocarcinoma, the most common form of cancer of the human lung. Other well-defined experimental models of lung carcinogenesis are also available, such as urethane was induced lung cancer [10],Therefore , this mouse model of lung tumor genesis is well suited for the identification of chemo preventive agents that could prevent lung cancer in current and former smokers [11] .

The second major cause of colorectal cancer ( CRC) is of the deaths from cancer in male and women in the world, Combined women; 2015 Statistical estimates show~132,700 new cases of a CRC and 49,700 related deaths[12]. Authors observed that colorectal cancer is at elevated risk due to various Having a cigarette may be a great number of carcinogens that can produce DNA damage and can cause irreversible damage in lung and Colorectal mucosa. [13] The present work aims to investigate the cytogenetically of tobacco and urethane using other animal models by other cytogenetic endpoints. induce and assess DNA damage by chromosome aberration and micronuclei .

## Material and Methods

The study is conducted on 30 males of laboratory white mice *Mus musculus* weighting 20-25g, 8-12 weeks age. Animals were maintained under controlled ambient temperature 25 C°, and a 12\ 12 hrs. light\ dark cycle for two weeks prior to commencement of the experiments.

**Chemical material**

Urethane crystals 5 mg/ml (ready to prepare),It was freshly prepared via dissolving in adequate volume of sterile distilled water to obtain the desired concentration. also used tobacco type (MIAMI m) (Nicotine 0.8 mg and 12 mg tar) treatments were for 16 consecutive weeks for all groups except negative control . after 24 hours of the last dose the animals were sacrificed in both tests.

**Experimental design**

In this experiment, the animals were treated with two intraperitoneal injections of urethane (5g/kg. body weight), with an interval of 48 hours. Water and food were given and libitum and no replacement of animals was done in case of death. Cigarette smoking experiment was performed in a closed chamber, once or twice a day, five days a week. The mice were randomly divided into six groups (G0, G1, G2, G3,G4and G5) as follows: G0 (n=5) control group received only water; G1 (n=5) received only urethane ;G 2 was exposed to the smoke of five cigarettes for 10 minutes twice a day leading to ten cigarettes in 20 minutes ; G3 (n=5) was exposed to the smoke of three cigarettes for 10 minutes twice a day, leading to six cigarettes in 20 minutes; and the G4 group (n=5) was exposed to five cigarettes for 5 minutes once a day; G4 group (n=5) was exposed to three cigarettes for 5 minutes once a day.

**Cytogenetic studies Micronucleus tests(MNi ):**

In Bone-marrow preparations were made and stained by May grunwald- Giemsa stain according to the method described in schmid [15]. Treatment induction test of micronucleus in bone marrow after injection interpreton administration of urethane 5mg/kg and tobacco inhalation the mice by exposure system for 10,5 minuet. The presence of micronucleated polychromatic erythrocytes was visually scored (1000 for bone marrow per mice) by optical microscopy using a Leica bright field microscope. Cells were considered to be micronucleated when they contained neatly defined chromatin corpuscles with diameter of less than one third the diameter of the cell nucleus and stained equal or denser than the nucleus of the cell from which the micronucleated cell had developed [16, 17].

**Chromosome aberration:**

In this study 30 males of mice were used, it was hard to obtain chromosomal spreads with therapeutic doses, therefore dose (5 mg.kg-1.bw of urethane) were used in this test, 5 animals for each group and treated as follow:

1. 5 animals as negative control treated with distilled water.
2. 5 animals as positive control treated with 5 mg.kg-1.bw of urethane.
3. 5 animals treated with tobacco five cigarettes for 10 minutes twice a day .
4. 5 animals treated with tobacco three cigarettes for 10 minutes twice a day
5. 5 animals treated with tobacco five cigarettes for 5 minutes once a day
6. 5 animals treated with tobacco three cigarettes for 5 minutes once a day

**Histological examination**

For histological examination, portions of the liver and lung were removed and preserved. The tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned. The sections were stained with haematoxylin and eosin.

**Exposure System**

Side stream smoke was created in a smoking chamber by burning cigarettes (MIAMI)$^{M}$. In a glass, the cigarettes were lightened and left to glow in the compartment and the smoke produced was drawn into the chamber where the mice were placed for exposure only. The atmospheric air in the chamber was checked for carbon monoxide (CO).[19]

Statistical.

The values were increased statistically using the statistical analysis software of the Statistical Package for Social Sciences (SPSS) and the findings were presented with the mean arithmetic mean ± standard

error. In order to detect substantial differences, the findings of the present analysis were statistically analyzed using the ANOVA test by comparing the statistical averages of the various experimental groups and using the TUKEY test.

## Result and Discussion

**Cytogenetically studies:** Micro nucleated MNi (Table 1, Figure (1) show increased in tobacco treated mice with the maximum MNi being induced by 5 cigarettes in 10 minutes dose. it about (29.40 +\- 3.71) (P≤ 0.05) while the other doses about Mean and S.D respectively about tobacco and urethane (3cig 10 minute, 5cig in 5 minute, 3cig in 5 minute, and urethane 5mg\kg .bwt) P≤ 0.05 respectively, (26.80 +\- 5.50 ) (23.06 +\- 3.05) , (22.20 +\- 5.07) (6.80+\- 5.50) in cells carrying intrinsic genomic instability and in normal cells exposed to genotoxic agents, MNi increased.

widely observed. compared to safe controls, urethane and tobacco can increase the rate of nuclear abnormalities in both smoking and smokeless types. the polychromatic erythrocytes PCE in the bone marrow, at early developmental stages,. As it matures, ratio decreases slowly. The ratio of PCE to MNi decreases when an animal gets exposed to toxic agent or genotoxic compounds either through environmental contamination or through the emergency medications[20]. polychromatic erythrocytes recorded in highest dose in 5cig 10mni it about (101.20 +\- 3.83) compartment with control group (16.06 +\- 0.5). also the other doses about (tobacco, urethane), (3cig 10 minute, 5cig in 5 minute, 3cig in 5 minute and urethane 5mg\kg .b.wt) P ≤ 0.05 respectively(32.09 +\- 1.2, 26.80 +\- 5.50, 23.60 +\- 3.05, 22.20 +\- 5.07) (Table 1). The present study assessed that the mean number of micronucleated and mean ratio of polychromatic to micchromatic erythrocytes (PCEs / MNi) of tobacco is clastogenic, causing a steady and highly significant increase in the mean number of Mni\PCEs, but causing a significant increase in mean PCEs compared with regulation. The micronucleus technique has been suggested as a useful method for genotoxicity calculation. [21].

In the metaphase chromosome analysis test (table 2, Figure 2) is one of the important tests that detect the extent of the drug's genotoxic effect on shape chromosomes, as the chromosomal abnormality is the same it is known that there are two types of numerical and structural chromosomal abnormalities, as studies indicate that there are mutations in Checkpoints in the cell cycle contribute to frequent errors during the phase Dissociation, which leads to a defect in the overall number of chromosomes. The anomaly appears Numerical chromosome[22] That the rate of replication of cells containing micronuclei following exposure The chemical is an indicator of clastogenic activity. As the micronuclei may also arise from the lagging chromosomes during mitosis. due to the loss of their connection to the spindle, the micronucleus test is able to identify chemicals that cause the spindle to malfunction, such as Colchicine and related spindle toxins[23] [24]indicate that however, the test material used is able to induce formation of micronuclei under test conditions as a result of chromosomal damage events (effective) Clastogenic and / or mitotic complex impairment [24] .

Figure (2) shows that urethane and tobacco cause structural chromosomal abnormalities such as fragments, gaps, deletions and robertsonian transmission within bone marrow cells, as shown in table (3), that the treatment by exposure to inhalation with intraperitoneal injection in the swiss white population caused differences. significant mean values and S.D of the sum of structural chromosomal anomalies, and the highest value of the mean sum of structural chromosomal anomalies was recorded in Tobacco treatment group reached. 5 cig.in 10m and urethane dose 5 mg/kg. body weight the structure aberration total with gap (31.00 +/- 2.00) and without gap (28.02 +/- 5.50), (24.20 +/- 2.83) also in other doses respectively for dose urethane 5mg/kg . body weight and tobacco dose 3cig 10 minute , 5cig in 5 minute, 3cig in 5 minute Structure aberration, total with gap Mean and SD (15.00 +/- 3.24 ) (27.40 +/- 1.67) (21.40+/- 1.67) (18.20 +/- 3.11) and without gap respectively (11.80 +/- 2.17) (21.00 +/- 1.58) (16.40 +/- 2.30) (14.00 +/- 2.06 ) P≤ 0.05 (Table 2). The importance of recording gaps in estimating the scalability of vehicles is controversial [25] finds the gaps unable to be considered chromated and chromosomes are synthetic chromosomal abnormalities unless the frequency of their observations is high. So in this case, an average calculation was made. Synthetic chromosomal anomalies in tropical phase brushes with gaps and no gap . The Fragments and breaks , the most common types of synthetic anomaly in the current cycle [26] between the emergence of fragments and/or fractures and between genetic mutations, he noted that these fragments that can be lost when cell division can be a source loss chromosome was inherited substance during division in the penta-cell, and the probability of this genetic loss may be difficult in the steadily divided cellular groups[26][27] .the cause of the emergence of patterns of calogenic abnormalities such as fragments, gaps, deletion, in pure cells the bone may be a direct chemical attraction to chromosomes. open fractures must be a sign of damage, which is represents the most important manifestation of genetic toxic exposure, as chromosomal fractures arise when exposure to phase G1 and chromatic fractures is limited when exposure is in phase S or G2 of the cell cycle. And that most of the mutation of chemicals are capable of one of the fragments and/or fractures in chromosomes .[28]

Study of histopathology ,classification as hyperplasia and nodules (Figure 3). In this model, chose to use this classification to validate the diagnosis of cancer, although the histological characteristics strongly

indicate that adenomas are the lesions found abnormal tissue [29].

The existence of alveolar cell proliferation without obliteration of more than four intra-alveolar spaces was the criterion for classification as hyperplasia; the nodule was intended for proliferation with obliteration of more than four intra-alveolar spaces. All the lesions were counted from both lungs. The lesions were subjected to histopathological examination and were graded as nodules and hyperplasia (Figure 3).

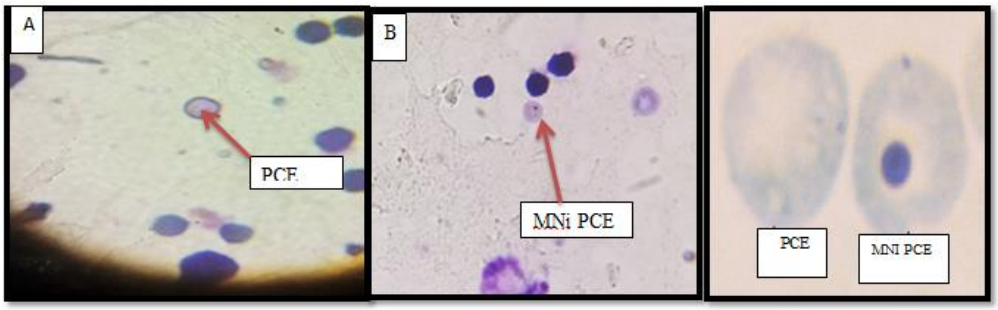



**Figure 1: An immature red blood cell containing MniPCE micronuclei inside the bone marrow of a mouse from the treatment group urethane and tobacco ( 100X Maygrünwald + Gemsa stain ) MNI= Micro nucleated, PCE= polychromatic erythrocyte (A) normal (B) treated with urethane .**

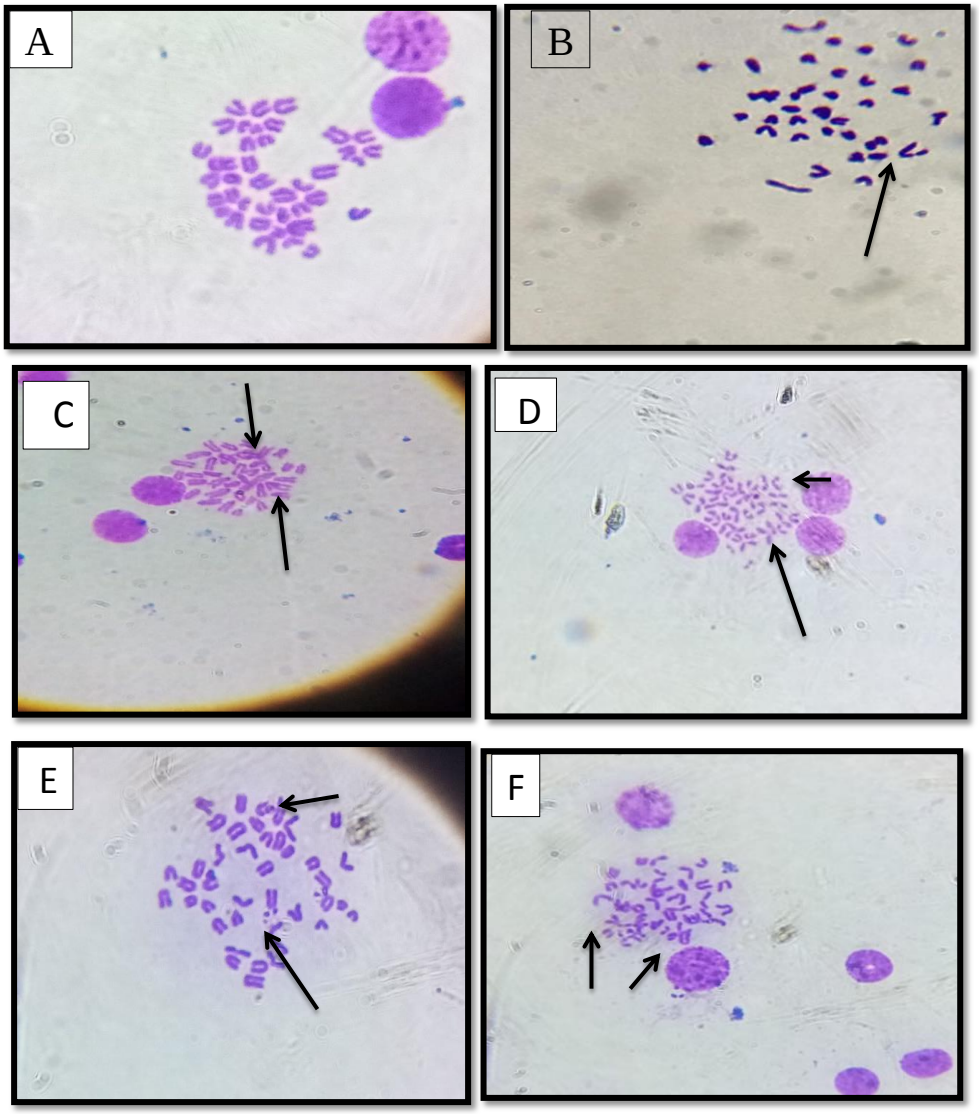
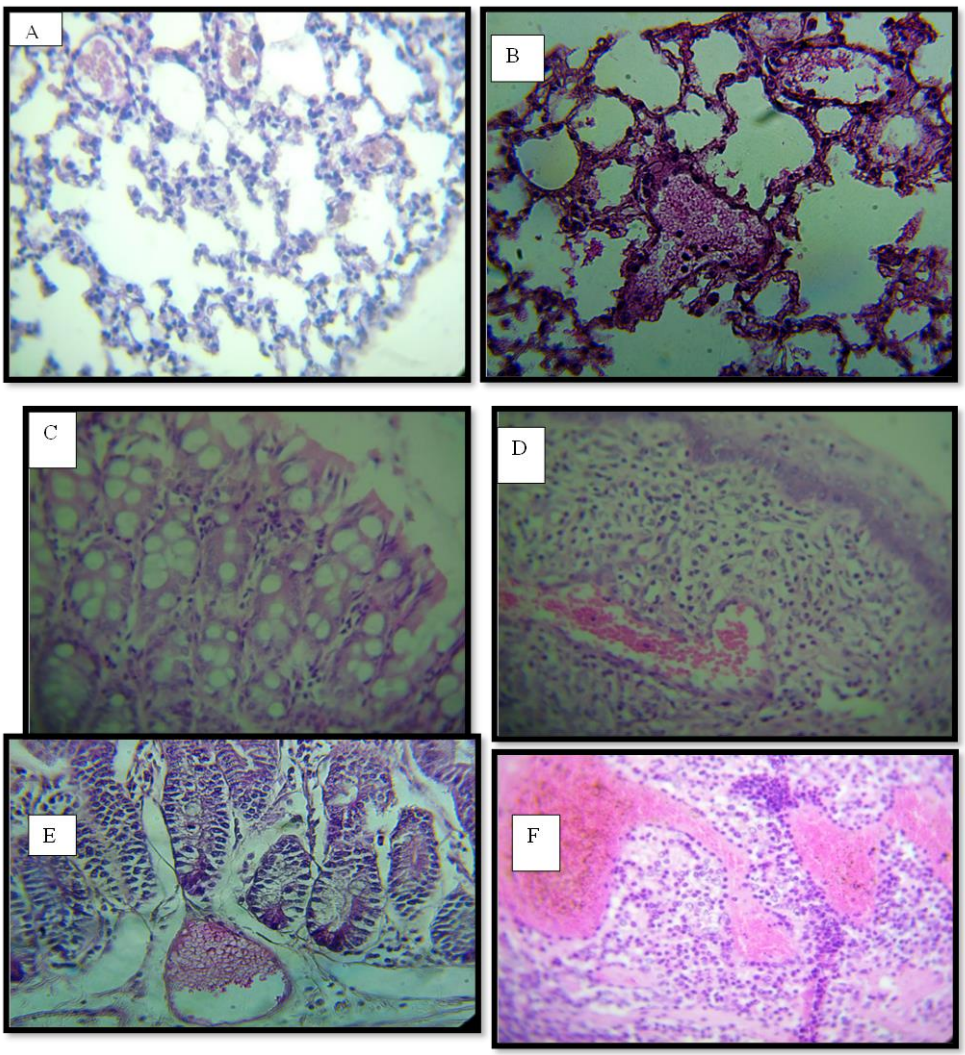



**Figure 2: Chromosomes of mice in bone marrow in stage metaphase , 100X n=40. (A) normal chromosomes (B) break (C) robertsonian translocation D) gap& deletion, (E) break & fragment, (F) deletion & fragment**




**(Figure 3)Photomicrograph of a representative in A normal lung tissue in B lung abnormal nodule and hyperplasia , in C normal colon tissue and D abnormal colon hyperplasia Inducer by tobacco ( 5 cig in twice day ) and urethane 5mg\kg. b.wt, in E abnormal colon ( tumor) hyperplasia of the cell , Lymphocytic infiltration with congestion of Blood vessels F abnormal lung ( tumor) hyperplasia of the cell increase congestion of Blood vessels , more Lymphocytic infiltration (A , B,C,D,E and F ) (H&E stain, 100x, respectively)**

**Table (1): Micronuclei in the bone marrow cells of the white Swiss R class after treatment with different doses of tobacco and negative control and positive in mouse bone marrow cells**

| Treatment mg.kg$^{-1}$ b. wt | Total Mni Mean+\- S.D | Total PCE Mean+\- S.D |
|---|---|---|
| **D.W** | 3.60 +\- 3.13* | 25.00+\-3.08* |
| **Urethane Ur** | **6.80 +\- 3.17** | 87.20+\-5.89* |
| **Tobacco and Ur 5cig 10mni** | 29.40 +\- 5.71 * | 101.20 +\- 3.83* |
| **Tobacco and Ur 3cig 10 minute** | 26.80 +\- 5.50 * | 97.80 +\- 3.70 * |
| **Tobacco and Ur 5cig in 5 mnit** | 23.60 +\- 3.05 | 95.55 +\- 3.43* |
| **Tobacco and Ur 3cig in 5mnit** | 22.20 +\- 3.07 | 89.77 +\- 3.30* |

*Significant at 0.01 * significant level at 0.05 paired sample T- test

**Table (2) Chromosomal aberration structure and numerical anomalies total with gap or without gap , treated with different doses of Tobacco and urethane .**

| Treatment mg.kg-1 b. wt | Numerical aberration Total Mean St.dev | Structure aberration Total With gap Mean St dev | Structure aberration Total without gap Mean St dev |
|---|---|---|---|
| D.W | **0.0 +/- 0.0** | **0.00 +/- 0.00*** | **0. 00 +/- 0.00** |
| Urethane 5mg/kg body weight | **1.600+/- 0.89 *** | **15.00 +/- 3.24*** | **11.80 +/- 2.17 *** |
| Tobacco and Ur 5cig 10mn | **12.20 +/- 1.09*** | **31.00 +/- 2.00 *** | **24.200 +/- 0.83*** |
| Tobacco and Ur 3cig 10 min | **11.20 +/- 3.10** | **27.40 +/- 1.67 *** | **21.00 +/- 1.58 *** |
| Tobacco and Ur 5cig in 5 mn | **9.30 +/- 4.06** | **21.40+/- 1.60 *** | **16.40 +/- 2.30 *** |
| Tobacco and Ur 3cig in 5mn | **9.05+/- 4.02*** | **18.20 +/- 3.11 *** | **14.00 +/- 4.06 *** |

*Significant at 0.01 * significant level at 0.05 paired sample T- test

**Table 3 : Cells with chromosomal aberration structure and numerical of chromosomal aberrations in white mice with different doses ,Tobacco and urethane induced .**

| MD±SD | Cells with chromosomal aberration | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Structure aberration | | | | | | Numerical aberration | | | |
| cross | Robertsonian | Fragment. | Deletion. | break | gap | total | Polyploidy | Aneuploidy | **Treatment Mg/Kg .bwt** |
| - | - | - | - | | -- | - | - | - | D.W |
| *0.40 +/- 0.54 | 2.40 +/- 0.89 | 2.00+/- 0.00 | 4.00+/- 1.00 | 3.00+/- 1.22 | 3.20 +/- 1.64 | *5.00 +/- 1.87 | 0.60+/- 0.54 | *4.40 +/- 1.81 | Urethane dose |
| *1.40+/- 0.54 | 3.80 +/- 1.92 | 5.40 +/- 0.89 | 5.80+/- 1.48 | 7.40+/- 1.14 | 6.80 +/- 1.30 | 10.80 +/- 2.77 | 1.00 +/- 0.70 | *9.60 +/- 2.41 | smoker 5 cig.in 10m + urethane dose 5 mg/kg |
| 1.80+/- 1.09 | 3.00+/- 1.22 | 4.60 +/- 1.34 | 4.80 +/- 0.83 | 6.80+/- 0.83 | 6.40 +/- 1.51 | 10.60 +/- 1.94 | 1.80 +/- 1.30 | 8.80 +/- 1.30 | smoker 3cig.in 10m + urethane dose 5 mg/kg |
| 0.80+/- 0.83 | 1.80 +/- 0.83 | 2.20 +/- 0.447 | 3.60 +/- 1.14 | 5.80+/- 1.92 | 5.80 +/- 2.59 | 9.20 +/- 2.86 | 0.60+/- 0.54 | 8.60 +/- 2.41 | smoker 5 cig.in 5m + urethane dose 5 mg/kg |
| 0.60 0.54 | 2.40 +/- 0.89 | 3.00 +/- 1.73 | 3.80+/- 1.09 | 5.00+/- 1.87 | 4.40+/- 1.51 | 8.20 +/- 0.83 | 1.00 +/- 0.70 | 8.60 +/- 0.89 | smoker 3 cig.in 5m + urethane dose 5 mg/kg |

*Significant at 0.01 * (significant at 0.05 level paired sample T- test)

## References

[1] El-Sokkary, G.H.; Cuzzocrea ,S. and Reiter, RJ (2007) Effect of chronic nicotine administration on the rat lung and liver beneficial role of melatonin. *Toxicology* 239: 60-67.

[2] Gawish , A.M. ; Ramadan, S. ,Hassan A.M. and Issa A.M.(2010). Morphometrical, Histopathological, and Cytogenetical Ameliorating Effects of Green Tea Extract on Nicotine Toxicity of the Testis of Rats. *Cytological Histological* , 17:347–357.

[3] Limburg, P.J., Vierkant, R.A. and Cerhan, J.R, (2010) Cigarette smoking and colorectal cancer : long-term, subsite-specific risks in a cohort study of postmenopausal women. Clinical Gastroenterol Hepatolal., **1(3)**:202–210.

[4] Schutze, M; Boeing, H; Pischon, T; Rehm, J; Kehoe, T; Gmel, G; Olsen, A; *et al*., (2011) Alcohol attributable burden of incidence of cancer in eight European countries based on results from prospective cohort study . 342: d1584.

[5] Sasco, A.J; Secretan, M.B; Straif, K .(2004): Tobacco smoking and cancer.a brief review of recent epidemiological evidence . *Lung Cancer*, 2:10.1016

[6] Malkinson AK. (2001) Primary lung tumors in mice as an aid for understanding, preventing, and treating human adenocarcinoma of the lung. *Lung Cancer* , 32:265–79.

[7] Janusz Datta, Paulina Kosiorek and Marcin Włoch .(2016). Synthesis, structure and properties of poly(ether-urethane) s synthesized using a tri-functional oxypropylated glycerol as a polyol J Therm Anal Calorim 10: 5928-2.

[8] Berenmblum I, Ben-Ishai D, Haran-Ghera N, Lapidot A, Simon E and Trainin N. (1989) Skin initiating action and lung carcinogenesis by derivatives of urethane (ethyl carbamate) and related compounds. Biochem Pharmacol 2:168-176.

[9] Jemal A, Siegel R, Ward E, *et al*. (2008) Cancer statistics,2008. CA Cancer J Clin 58:71–96.

[10] Parajuli R, Bjerkaas E, Tverdal A, Selmer R, Le Marchand L, Weiderpass E, Gram, IT.(2013) increased risk of colon cancer due to cigarette smoking may be greater in women than men. *Cancer Epidemiol Biomarkers* 22:862–871.

[11] Huang K, Sandler RS, Millikan RC, *et al.* (2006) GSTM1 and GSTT1 polymorphisms, cigarette smoking, and risk of colon cancer: apopulation-based case-control study in North Carolina .*Cancer* 17:385–394.

[12] Luchtenborg M, White KK, Wilkens L, *et al.* (2007) Smoking and colorectal cancer: different effects by type of cigarettes. *Cancer Epidemiol Biomarkers* 16:1341–1347.

[13] Fekadu Kassie, Ilze Matise, Mesfin Negia, Pramod Upadhyaya, and Stephen S. Hecht (2008) Dose-Dependent Inhibition of Tobacco Smoke Carcinogen–Induced, Chemoprevention of Lung Tumorigenesis by Indole-3-Carbinol Lung Tumorigenesis in A/J Mice by Indole-3-Carbinol .

[14] Paget, G. F. and Barnes,J.M. (1964). Toxicity testing in Evaluation of Drug Activities. In. Laurence. D. R. and Bachrach, A. L. (eds.) pharmacometrics. Academic press, London: p .161.
[15] Schmid, W. (1986). The Cell Micronucleus Test for Cytogenesis analysis. In: Chemical Mutagens Principles and Methods for their Detection. 4. Hollaender, A. (Ed.). Plenum Press, New York & London. pp: 31-53.
[16] Abramsson, Z.L.(1997). Chromosome aberration detectied by the flow cytometer based micronucleus assay . Acta Univ.Ups.278. pp:109-113.
[17] Ni, Z.S. *et al*. (1993). Induction of micronucleus by organophosphorus pesticides both in vivo and in vitro. 24: 82-86 .
[18] Cury PM, Lichtensfels AJFC, Reymão MSF, Conceiçao GMS, Capelozzi VL and Saldiva PHN. (2000) Urban levels of air pollution modifies the progression of urethane-induced lung tumors in mice. Pathol Res Pract 196:627-633.
[19] Berenmblum I, Ben-Ishai D, Haran-Ghera N, Lapidot A, Simon E and Trainin N. (1959) Skin initiating action and lung carcinogenesis by derivatives of urethane (ethyl carbamate) and related compounds. Biochem Pharmacol . 2:168-176.
[20] Walum, E. (1998). Acute Oral Toxicity. Enveronmental Health Perspectives. vol.106 Supplement 2.
[21] Stinn W, Teredesai A, Kuhl P, Knorr-Wittmann C, Kindt R, Coggins C and Haussmann HJ. (2005) Mechanisms involved in A/J mouse lung tumorigenesis induced by inhalation of an environmental tobacco smoke surrogate. Inhal Toxicol. 17:263-276.
[22] Yadav, V.P., Sahoo, N. R., Kumar, P., Gaur, G. K., Sahoo, A. P, and Saini, B. L. (2018) Cytoscreening of Indigenous pigs for Numerical Chromosomal Abnormalities. J Pharm In nov, (7)pp: 14-17.
[23] El-Sokkary, G.H. ( 2007) . An autoradiographic study of cellular proliferation, DNA synthesis and cell cycle variability in the rat liver caused by phenobarbital-induced oxidative stress: the protective role of melatonin. Cell. Mol. Biol. Lett. 12, 317–330.
[24] Krishna, G. and Hayashi, M.(2000). In vivo rodent micronucleus assay: protocol, conduct and data interpretation. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis.455(1-2) :155–166.
[25] Yildiz, D., Liu, Y.S., Ercal, N., Armstrong, D.W., (1999). Comparison of pure nicotine- and smokeless tobacco extract-induced toxicities and oxidative stress. Arch. Environ. *Contam. Toxicol*. 37, 434–439.
[26] AL-Husni, M. A. (2003). Quail production, First Edition , Ibn Sina Press, Cairo. pp :5 -8.
[27] Salama, S. A.; Fahmi ,A. I. and Abo El-Ghar , G. E. S. (1995). Chromosomal Aberrations and Sperm head Abnormalities Induced by Abamectin (Avermectin B1) and its Degradates in Male Swiss Albino Mice. The Japan Mendel Society Cytologia. 60:411-417.
[28] Stinn W, Teredesai A, Kuhl P, Knorr-Wittmann C, Kindt R, Coggins C and Haussmann HJ. (2005)Mechanisms involved in A/J mouse lung tumorigenesis induced by inhalation of an environmental tobacco smoke surrogate. Inhal Toxicol 17:263-276.
[29] De Flora S, Balansky RM, D'agostini F, Izzotti A, Camoirano A, Bennicelli C, Zhang Z, Wang Y, Lubet RA and Ming You. (2003) .Molecular alterations and lung tumors in p53 mutant mice exposed to cigarette smoke. *Cancer* .63:793-800.

# دراسة تأثير السمية الوراثية والخلوية لليوريثان والتبغ في الرئة والقولون والمستقيم للفئران البيضاء

رنا نصير عبدالحميد ، وجدي صبيح صادق ، عادل فوزي شهاب

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**الملخص**

تم تقدير التأثيرات السمية الوراثية والخلوية لعقار الكيميائي اليوريثان والتبغ في نقي عظم الفئران السويسرية البيضاء *Mus musculus* بوساطة اختبار تقدير النواة الدقيقة Micronucleus test(MN) في كريات الدم الحمر غير الناضجة متعددة التلوين Polychromatic Erythrocytes (PCEs) وتحليل كروموسومات الطور الاستوائي لتقدير الشذوذ الكروموسومي Chromosomal aberration analysis . في هذه الدراسة ، تم تقييم التأثيرات السامة للجينات والسمية للخلايا للتبغ واليوريثان في نخاع العظام للفئران السويسرية البيضاء باستخدام اختبار النواة الدقيقة (MNi) في كريات الدم الحمراء متعددة الألوان (PCEs) ، تحليل انحراف الكروموسوم. في هذه الدراسة ، تم فحص تأثير الجرعات المختلفة من دخان السجائر وعقار اليوريثان، كذلك دراسة التغيرات الشكلية (المورفولوجية) في كل من الرئة والقولون والمستقيم في الفئران. بعد حقن 5 ملجم / كجم من يوريثان، تعرضت الفئران بعد ذلك لدخان التبغ مرة أو مرتين يوميًا، خمس مرات في الأسبوع ، في غرفة مغلقة. تم تقسيم الحيوانات عشوائيا إلى ست مجموعات، بينما اعطيت مجموعة السيطرة السالبة الماء المقطر فقط. أشارت نتائج التحليل الإحصائي إلى زيادة معنوية في $P\leq0.05$ من خلايا الدم غير الناضجة ذات النوى الدقيقة في نخاع العظم لكل من عقار اليوريثان والتبغ في تكوين النوى الصغيرة مقارنة بمجموعه السيطرة السلبية. أشارت نتائج الدراسة أيضًا إلى زيادة معنوية في $P\leq 0.05$ في تحريض الشذوذ الكروموسومي لخلايا الدم الحمراء غير الناضجة في نخاع العظام. كما أظهرت النتائج عدد العقيدات والتضخم بين المجموعات المختلفة التبغ والتغيرات النسيجية التي يسببها اليوريثان في كل من أنسجة الرئة والقولون والمستقيم كما أظهرها المجهر الضوئي ، وكشفت الدراسة الحالية أن التبغ واليوريثان لهما تأثيرات سامة وراثية على الخلايا الجسدية للفئران البيضاء ، وكذلك المدخنون أكثر عرضة للإصابة بسرطان الرئة والقولون.