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CYTOGENETIC AND BIOCHEMICAL EFFECTS OF INCENSE BURNING ON LABORATORY MICE

G.M.Hassan[*], A.S.Hassan[**]., A.A.Yassein[*] and Magda Ragab[***]
[*] Genetics Dept., [**] Biochemistry Dept., [***] Food Sciences and Technology Dept., Fac. Agri., Fayoum Univ., Egypt.

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ABSTRACT

Incense smoke is a potential hazard to human health due to various airborne carcinogens emitted from incense burning. The present study was conducted to determine the genotoxic potential health effects of exposure to organic compounds emitted from incense burning. Chromosomal aberrations in bone marrow cells showed that incense burning generates significantly higher levels of metaphase abnormalities and a significant increase in DNA damage in treated groups compared to those of the control. Also, found that the activity of serum lactate dehydrogenase (LDH) increased significantly in incense smoke exposure groups compared to control. These results indicate that exposure to carcinogens emitted from incense burning may increase health risk.

INTRODUCTION

Incense has been used since ancient times to produce pleasant fragrances or to mask odors, and incense burning has been incorporated in many religious ceremonies and practices. Burning of incense is common in Arabian households and places of worship, often in confined, poorly ventilated places. Incense is also burned to repel mosquitoes, therefore, there is continuous exposure to incense smoke in many individuals, and incense emissions are thus an important source of indoor air pollution that may be associated with respiratory disorders or increased risk of cancer. An epidemiological study demonstrated an association between chronic use of incense and squamous-cell carcinoma of the respiratory tract **(Friborg *et al.*, 2008)**. Incense was also reported to be linked to leukemia in children **(Roveri *et al.*, 1998 and Jetter *et al.*, 2002)**.

Burning of incense would emit mixture of gases and particles. The gaseous phase includes volatile organic compounds, aromatic aldehydes, aliphatic aldehydes **(Yang *et al*., 2006)**, and polycyclic aromatic compounds (PAHs) **(Lin and Lee, 1998)**. Gaseous PAHs and aliphatic aldehydes detrimentally affect health. Thus, adverse health effects with respect to the exposure to incense smokes may be as a result of the combination of extremely high number of sub micrometer particles and organic compounds occurring in those smokes **(Lee and Wang, 2004)**. An incense risk of leukemia was found for children whose parents burned incense in the home before pregnancy or during the nursing period **(Lowengrat *et al*., 1987)**. **Fan and Zhang (2001)** found that, burning one gram of incense produce 180- 220 and 0.8-1.1 mg of carbon monoxide and isoprene, respectively.

Incense smoke was also found to be mutagenic to ***S. typhimurium*** TA98, TA100 and TA104 in the presence of exogenous activation system,however, no mutagenic activity was detected in extracts of unburned incense **(Rasmussen, 1987)**. Incense smoke not only contains oxidative mutagens, but also can cause frame shift mutation **(Chang *et al*., 1997)**.

Incense smoke contains several different mutagenic species, but they do not permit identification of any particular one as being prominent. No mutagenic activity was detected in extracts of unburned incense, clearly indicating that the mutagenic compounds were formed during the burning and were not present as constituents of the incense itself. **(Rasmussen, 1987)**.

Incense smoke condensates caused genotoxic effects such as induction of sister chromatid exchange, and the genotoxicity of certain incense smoke condensates in mammalian cells seemed to be higher than those of tobacco smoke condensates **(Chen and Lee, 1996)**.

Oxidative DNA damage is also related to the secondary effect of PAHs via generation of reactive oxygen species (ROS). DNA damage without effective repair may produce cellular dysfunction and diseases, such as cancer **(Hsieh and Yamane, 2008)**. It was found that burning incense generated high concentrations of ROS in the particulate gas phase of the emissions **(Kao and Wang, 2002)**. These ROS may damage DNA and other biomolecules through 8- hydroxy-2-deoxyguanosine which induce oxidative DNA damage and DNA strand breaks **(Liu *et al*., 1996)**. ROS- induced oxidative damage can also alter the defense mechanisms such as, the antioxidant system and DNA repair process. A number of studies indicate oxidative damage as an important factor in cancer development including initiation, promotion and progression stages of

carcinogenesis **(Navasumrit *et al.*, 2008)**. This study was conducted to determine the effects of exposure of mice to incense smoke on mice chromosomes, DNA and some serum enzymes.

MATERIALS AND METHODS

Incense tested

Commercial powder incense purchased from a local market in Fayoum, Egypt, was tested in this Study.

Chamber experiments

The controlled experiments were conducted in a 2 x 2 x 2 m^3 aluminium environmental test chamber, the air exchange was maintained by used a fan to simulate the typical natural ventilation conditions. The source of burning was located outside of the chamber to avoid the effect of temperature, the incense smoke was entered to chamber through a plastic tubes by pump located outside the chamber.

Test animals

An inbred strain of swiss albino male mice, weighting 22-25 g. were obtained from the Animal House of the Faculty of science, Fayoum University, Egypt and maintained at a temperature of 25±2 ºC. The mice were provided with standard pelleted feed and tap water *ad-libitum*. Healthy mice were selected and distributed to four groups (6 mice / group) as follows: group 1, control, group 2, exposed to incense smoke for 5 minutes, group 3, exposed to incense smoke for 10 minutes and group 4 , exposed to incense smoke for 15 minutes for eight weeks.

Preparation and analysis of chromosome aberrations

Twenty-four hours before sacrifice, animals in all groups were given a suspension of yeast powder (100 mg/500 ul) to accelerate mitosis of bone-marrow cells. For chromosomes preparations, each mice was injected with 0.1ml of 0.025% colchicine solution intraperitoneally for one hour and 30 minutes before sacrifice in order to block dividing cells in metaphase.

Bone-marrow cells from femurs and tibia were flushed in 1% sodium citrate solution at 37ºC and fixed in acetic acid / ethanol (1:3). Slides were prepared by the conventional flame drying technique followed by Gimsa staining **(Khuda *et al.*, 2002)**. After coding of the slides, the chromosomes of 100 cells in metaphase were examined for abnormalities at a magnification of 1000X using an optical microscope

(carl Zeiss). This was done for each one of three replicates (300 metaphases) for control and treatment groups. Chromosome aberrations were identified according to criteria described by **Savage, (1975)**. Metaphases with chromosome break, gaps, rings, and fragments were recorded as percentage of total metaphases per group and photographed.

DNA isolation from blood

Genomic DNA was isolated from blood by potassium ethyl xanthogenate (XS) buffer according to **Tillett and Neilan (2000)**.

Serum enzymes

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically using the procedures of the method of **Reitman and Frankel (1957)**. Total lactate dehydrogenase (LDH) activity was assayed in serum as described by **king (1965)**.

GC-MS analysis of incense burning

The smoke sample was analysed by a gas-chromatography at plant pathology institute, Agriculture research centre, Giza, Egypt.

Statistical analysis of data

Analysis of variance was computed using the General linear Model procedure of statistical analysis system **(SPSS, 1997)**. Variable means for treatments indicating significant differences in the ANOVA were compared and, the differences were indicating using Duncan multiple range tests **(Duncan, 1955)**.

RESULTS AND DISCUSSION

Chromosomal aberrations

Table (1) shows the results of the chromosome aberration assay in the control and treated groups. (Figs. 1-6) shows representative photographs of the metaphases with normal and aberrant chromosomes. Incense smoke treatment appeared to result increase in aberrant metaphases in three treatment groups with increase in chromosome aberrations of the third treatment group (15 min. exposure), being significantly different (42.67%) compared to the control group (2.00 %) ($P < 0.05$). Ring chromosome was shown to be the most frequent type of aberration followed by chromatid breaks and end to end association, while chromatid gaps were observed to be less frequent in this study and polyploidy was observed only in the group exposed to smoke for 15 min. (Fig.6). However we observed an exposure time dependent chromosome aberration frequency.

The present results are similar to **DeRaat ,(1979)** who mentioned that cigarette smoke can cause a chromosomal aberrations such as gaps, breaks, deletions, fragments and polyploidy in bone marrow cells of mice.

The increase in chromosomal aberration was dependent on exposure time to tobacco smoke **(Lee, 1996)**. The occurrence of chromosomal aberrations especially chromatide breaks, would indicate the smoke possibly acted after reproduction of chromosomes at G2 or late S-phase of DNA synthesis **(Biswas *et al.,* 2004)**. The other aberrations in the present study may be due to the existence of some weaker or relatively more vulnerable spots on the chromosomes. The mechanism by which mutagenic components which omitted from incense burning including resins, aromatic substances, essential oils and synthetic chemicals induces these genotoxic effects is due to the generation of reactive oxygen species during the biotransformation process leading to oxidative damage to DNA **(Shen *et al*., 1996)**.

Table (1): The main values of different chromosomal aberrations in bone marrow cells for mice exposed to incense smoke at different time, min. (Mean ± SE)

Items / Treatments (Exposure time, min)	Metaphase	Break	Ring	Gap	Frag	Del	End-End	Polyploidy
Control	2.00± 0.58^{B}	0.00± 0.00^{b}	0.67± 0.33^{C}	0.00± 0.00^{a}	0.00± 0.00^{b}	0.00± 0.00^{a}	1.33± 0.33^{c}	0.00± 0.00^{a}
5	11.33± 1.85^{B}	0.67± 0.33^{b}	6.33± 0.88^{BC}	1.00± 0.47^{a}	0.67± 0.33^{b}	0.33± 0.33^{a}	2.33± 0.33^{bc}	0.00± 0.00^{a}
10	34.00± 4.00^{A}	5.67± 1.20^{a}	16.00± 0.58^{AB}	0.33± 0.33^{a}	4.67± 0.58^{a}	1.33± 0.33^{a}	6.00± 1.15^{a}	0.00± 0.00^{a}
15	42.67± 9.60^{A}	7.33± 2.40^{a}	27.33± 3.06^{A}	0.67± 0.47^{a}	1.33± 0.88^{b}	1.67± 0.67^{a}	4.33± 1.20^{ab}	0.33± 0.33^{a}

a. c and A. C. values in the same column within the same item followed by different superscripts are significantly different at $P<0.05$ for a to c ; $P< 0.01$ for A to C. Break: chromatid break, Ring: chromosome ring, Del: deletion, End- End: end to end association.

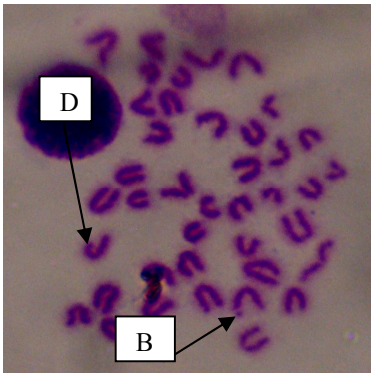

Fig.1

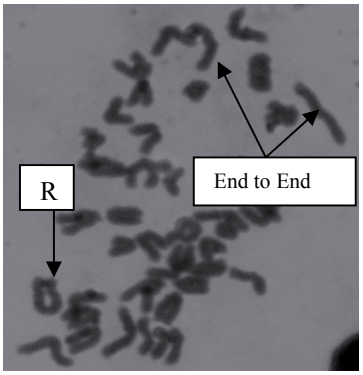

Fig.2

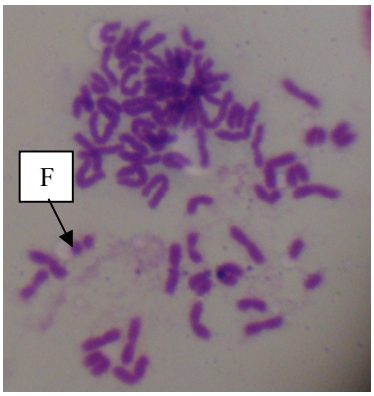

Fig.3

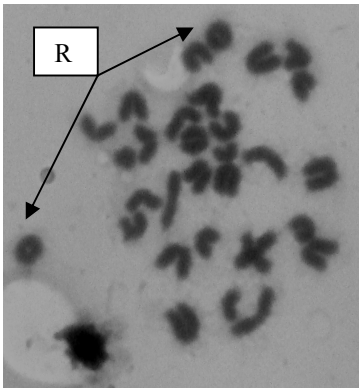

Fig.4

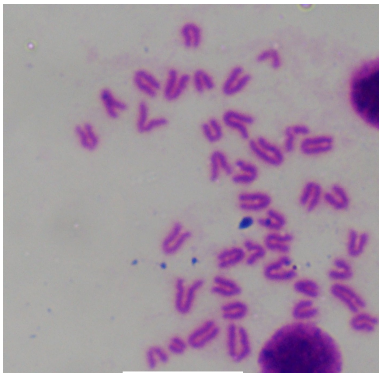

Fig.5

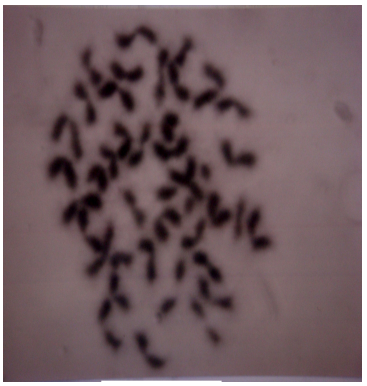

Fig.6

Fig. (1-6): Photomicrographs of incense smoke induced chromosomal aberrations In bone marrow cells of mice: a typical normal spread (Fig.5), spread with ring (Fig.2,4) ; chromatide break and deletion (Fig.1); end to end association (Fig.1); fragment (Fig.3); and polyploidy (Fig.6).

Effect on DNA fragmentations

Fig (7) shows the agarose gel electrophoretic pattern of DNA from blood in control and treated mice groups .DNA in control (group 1) mice yielded intact DNA. Smoke exposed (groups 2, 3 and 4) mice showed DNA fragmentations as evident by laddering pattern characteristic. DNA damage was also reported by **Navasumrit *et al.* (2008)**, they found that temple worker when were exposed to incense smoke had a significant increase in DNA damage and strand breaks, and they found that 8-hydroxy-2-deoxyguanosine (8-OHdG) represents an important product of oxidative damage to DNA induced by the reaction of hydroxyl radicals with guanosine at the C_8 site in DNA. A significant correlations between DNA damage and exposure levels to benzene and PAHs suggested that increased DNA damage my be in part due to the genotoxic effects of compounds generated from incense burning **(Chang *et al.*, 2007)**. The high biological relevance of 8-OHDG is due to its ability to induce G-T transversion, which is amongst the most frequent somatic mutations found in human cancer **(Chang *et al.*, 2007)**.

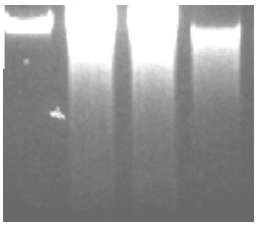

Fig.(7): Agarose gel electrophoretic pattern of DNA in control and treated . 1: control, 2: exposed to smoke for 5 min, 3: exposed to smoke for 10 min. and 4: exposed to smoke for 15 min.

Serum enzymes.

The total activity of LDH, AST and ALT in serum of control and experimental mice is shown in Fig (8) a significant increase ($P<0.05$) in the activity of LDH in serum was observed in group 4 mice exposed to smoke compared to control, whereas no significant changes

were observed in groups 2 and 3 mice compared to control. In addition there were no significant differences in the level of activities of AST and ALT enzyme in treated compared to control (Fig. 8). Incense smoke is a complex, oxidizing milieu possessing an array of free radicals and reactive oxygen species, which include hydroxyl, peroxyl and nitric oxide **(Pryor, 1997)**. Cell membranes being primarily composed of lipids especially polyunsaturated fatty acids are particularly susceptible to attack by permeability and altered fluidity of the membrane and thereby causing cellular leakage **(Cross *et al.*, 1987)**. In the present study, incense smoke exposure resulted in a significant elevation of LDH in serum, which may be due to the leakage of this enzyme from the necrotic tissues into circulation.

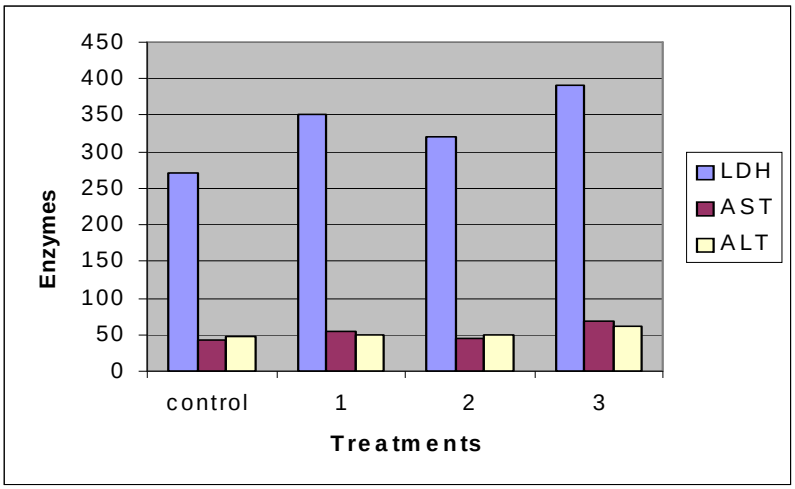

Fig. (8): Effects of incense smoke on mice serum enzymes (LDH, ALT and AST, unit /l).

Chemical analysis of incense smoke

Chemical analysis of volatile organic compounds in incense smoke using GC/MS indicated the presence of several polycyclic aromatic hydrocarbons (PHA), in addition to 1, 3-butadiene and furan compounds,Table (2). Base on the carcinogen classification by the international agency for research on cancer (IARC), the result show that 1, 3 butadiene and furan which are probable human carcinogen would be produced during burning of incense. The present results are similar to **Wang *et al.* (2007)**. In addition diethyl phthalate and some benzyl compounds which were reported by **Madany ,(1994)** were also confirmed by our experiments. Hence, incense burning should be avoided indoors as possible.

Table (2): The compounds present in the incense smoke

peak	Compounds	% of total
1	m-Di fluorotetrachlorobenzene	19.912
2	Diethyl phthalate	0.525
3	2,5furandion, 3-(2-dodecenyl)dihydro-	0.530
4	3-methyindole-2-carbaldehyde	0.547
5	cyclohexanone	0.421
6	Benzene,1,2-dichloro	0.281
7	Benzene,1,2,4-trichloro	0.359
8	1,3-butadiene	1.679
9	Benzene, 1.3,5 trimethyl	0.577
10	1,2-Benzenedicrboxylic acid,bis2-ethylhexylester	74.95
11	3-methylcyclopentadecan1,5-Dione	0.219

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الملخص العربى

التاثيرات السيتولوجيه والكيموحيويه للدخان الناتج عن احتراق البخور على فئران التجارب المعملية

جمال محمدين حسان* ، **عبد المنعم صادق حسن****، **أحمد عبد الفتاح محمد يسن***، **ماجدة رجب*****
* قسم الوراثة،** قسم الكيمياء الحيوية ،*** قسم علوم تكنولوجيا الاغذيه
كلية الزراعة - جامعة الفيوم - مصر

تم اجراء هذه التجربة لدراسة تاثير الدخان الناتج عن احتراق البخور على التغيرات الكروموسومية وكذللك تاثيره على الـ DNA و بعض انزيمات الدم لفئران التجارب المعمليه، تم تقسيم الفئران الى اربع مجاميع كل مجموعة بها 6 فئران وتم تعريض الفئران للدخان لمدة 8 اسابيع على فترات زمنيه مختلفه (0،5،10،15 دقيقة/ يوم) . وقد اظهرت النتائج اختلافات معنويه فى عدد الكروموسات الشاذه فى الافراد المعرضه للدخان بالمقارنه بالكنترول، كذلك وجد تأثير ملحوظ على تحلل الدنا المعزول من الفئران المعامله. و بمقارنة النشاط الكلى للانزيمات LDH , AST , ALT للمجاميع المعرضه للدخان بالكنترول وجدت زيادة معنويه فى نشاط انزيم LDH بينما لم تكن هناك تغيرات معنويه فى نشاط انزيمى AST, ALT .