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Preliminary Studies on General and Genetic Toxicity of Incense (Bakhour) Inhalation in Swiss Albino Mice

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Abstract: The investigation on general and genetic toxicity of Bakhour smoke inhalation in Swiss albino mice was undertaken in view of the literature reports on adverse effects of incense smoke and a paucity of literature on the general and genetic toxicity of Bakhour. The present study was conducted on both the sexes of Swiss albino mice. Treatment included exposure to a dose of 115 gram Bakhour burning smoke for a period of 2 hour, daily for 7 days. The observations on toxicity involved; behavioral changes (autonomic responses, motor activity and central nervous system), effect on body and organ weight and rate of mortality. Micronucleus test was used to determine the genetic toxicity. The different parameters studied were subjected to statistical analysis with one way ANOVA and Post hoc Tukey-Kramer multiple comparison test. The sub-acute treatment with Bakhour smoke failed to induce any significant changes in the symptoms of general toxicity as compared to the control. The animals remained calm during the treatment. There was moderate increase in respiration and mild increase in pilo erection and allergic symptoms. There was no mortality and effect on body weight. But weight of lungs altered significantly. The treatment failed to affect the frequency of micronucleated-polychromatic erythrocytes (PCE) and the ratio of PCE/normochromatic erythrocytes (NCE). The results of this preliminary report showed that Bakhour smoke is non- toxic. Nevertheless, there is a need for more extensive studies that include analyzing composition of Bakhour smoke, comparing the smoke of Bakhour with that of the smoke from other sources, in addition to extensive studies on chronic toxicity.

Key words: Bakhour % Incense % Smoke % Toxicity % Genetic Toxicity

INTRODUCTION

The term incense refers to the substance that release fragrant smoke when burnt. The content of the smoke appears to depend on the source and constituents of incense [1]. Bakhour is one of the many types of incense that is commonly used in most of the Gulf countries, including Saudi Arabia. It is the Arabic name given to scented bricks or a blend of natural traditional ingredients (oudh, woodchips). According to a local tradition in Saudi Arabia, Bakhour is burnt and the resultant smoke is involuntarily inhaled. Literature reports suggest Bakhour to contain only natural substances - musk, patchouli, bergamot, agar wood, frankincense, galbanum, cloves, hyacinth, oak, oak moss, jasmine, saffron, cardamom, cedar, amber, musk, cinnamon, sandalwood and shamamatul. Burning of Bakhour in charcoal or incense burners emanate smoke that is composed of aromatic biotic material. The fragrance of Bakhour is used as a gesture of hospitality on special occasions like religious ceremonies, weddings, receptions and for creating a mood and masking bad odors. The Incense fragrances can be of such great strength that they abstruse other un-desirable odors. This is also used in funerary ceremonies because the incense could oppress the scent of decay [1-3].

There are various forms of incenses, including sticks, joss sticks, cones, coils, powders, rope, rocks/charcoal and smudge bundles. Generally there is a base onto which the mixture of incense ingredients are attached [4]. While the exact content of incense sticks is a commercial secret, most incense is made from a combination of fragrant gums, resins, wood powders, herbs and spices [5]. The source of incense smoke depends on the raw materials used in the manufacture and incense from most of the sources is reported to contain various contaminants including gaseous pollutants, such as CO, NO_2, SO_2, volatile organic compounds, absorbed toxic pollutants (PAHs and toxic metals) [6]. In the particulate

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matter from incense smoke, particles less than 2.5 μm in diameter are referred to as fine particulars and are believed to pose the largest health risks because they can penetrate as deep as the alveoli [7]. It is presumed that the contaminants of incense smoke can cause respiratory diseases and are carcinogenic [3, 8].

In a study on analysis of ambient air measurement of a shrine, it has been found that incense burning generates significantly higher levels of airborne benzene, 1,3-butadiene and total PAHs compared to those of the control work place. The most abundant PAHs were chrysene, B[ghi]P, B[a]P, B[a]F and fluoranthene. Investigations on DNA damage and DNA repair capacity revealed that the shrine workers had a significant increase in DNA damage observed as a 2-fold increase in the levels of leukocyte 8-hydroxy-2'-deoxguanosine (8-OHdG) and DNA strand breaks. These results indicate that exposure to carcinogens emitted from incense burning may increase health risk for the development of cancer in shrine workers [9].

In a study on PAHs pollution survey conducted in the environment of public places in Hangzhou, China, Lu *et al*. [10] reported most serious PAHs pollution observed in indoor air of shopping centers, where the possibility of exposure to incense was more rampant. In shopping centers, supermarkets, shrines, the emission of PAHs occurred from indoor air mainly originated from incense burning. Health risks associated with the inhalation of PAHs were found to be exorbitant and greatest contribution to total health risks was from naphthalene. Chiang and Liao, [11] reported exposure to smoke emitted from heavy incense burning may promote lung cancer risk.

Lofroth *et al*. [12] investigated the emission of aerosol particles and their mutagenic activity and the emission of some gaseous pollutants, in order to compare the emission from some indoor pyrolysis processes. The authors found that incense and mosquito-coil burning can cause indoor air pollution similar to that from cigarette smoke. Their smoke and extracts were mutagenic in the Ames Salmonella test with TA98. Carbon monoxide, isoprene and benzene were present in the emissions from the smoke and burning processes. Indoor air pollution has now been recognized as a potentially important problem for public health. Due to the slow and incomplete combustion, the incense burning produces a continuous flow of smoke. The pollutants (toxic gases, PAHs, CO, benzene and isoprene) that generate are accumulated indoors, especially, when there is no proper ventilation [13]. Exposure to the smoke from incense is reported to cause illnesses, respiratory distress, asthma, elevated cord blood IgE levels, contact dermatitis, leukemia, brain tumor in children and adults and other types of cancer [13-15].

The impact of air pollution on human health has been recognized over the last several decades and mainly these smokes are known have deleterious effects on the respiratory system. In a study on Bakhour smoke, Alarifi *et al*. [14] found ultra structural changes in the Alveolar pneumocytes of Bakhour-exposed animals. These changes involved the cell organelles and surfactant material of type II cells. Hyperplasia of alveolar cells was a feature in the affected lung tissue. Neutrophils accompanied with degenerative and necrotic changes were found infiltrating pulmonary alveoli and of the alveolar cells. There was a thick deposition of collagen fibrils in the alveolar walls. Al-Rawas *et al.* [16] also found Arabian incense (bakhour) burning to adversely affect respiratory health. Nevertheless, there has been no study on the general and genetic toxicity of smoke emanating from Bakhour.

In view of the widespread use of bakhour in Arabian countries in general and Saudi Arabia in particular and the reported toxicity and probable carcinogenicity of incense smoke, it was found worthwhile to study the general and genetic toxicity of Bakhour smoke.

MATERIALS AND METHODS

Both the sexes of Swiss albino mice, aged 6-8 weeks, weighing 25-30 g were used. The animals were maintained in the animal facility of the College of Pharmacy, under standard conditions of temperature (23±1°C), light/dark cycle (12 h/12 h) and relative humidity. Treatment included exposure to a dose of 115 gram of Bakhour burning smoke for a period of 2 hour, daily for 7 days. This dose was determined safe on the basis of a preliminary experiment. The observations on toxicity [17] involved (i) behavioral changes (autonomic responses, motor activity and central nervous system) (ii) effect on body weight (iii) effect on organ weight and (iv) rate of mortality. Micronucleus test was used to determine the genetic toxicity. The procedure of micronucleus test described by Aleisa *et al.* [18] was followed. Thirty hour after the last exposure to Bakhour smoke, the animals were sacrificed. The marrow aspirated in fetal calf serum was centrifuged and smears were made from the residual cells. After air-drying, the slides were fixed in methanol and stained in May-Gruen Wald, Giemsa and water. The markers of study were micronuclei in polychromatic erythrocytes (PCE) and the ratio of PCE and normochromatic erythrocytes (NCE). The different parameters studied were subjected to statistical analysis with one way ANOVA and Post hoc Tukey-Kramer multiple comparison test.

RESULTS

The sub-acute treatment with Bakhour smoke failed to induce significant changes in the autonomic responses, except moderate increase in respiration in both male and female mice, as compared to the animals in the control group. There was mild pilo erection observed in both the sexes. No changes in motor activity, as compared to the animals in the control group were observed. The category of CNS excitation revealed mild allergy, showing symptoms of itching in both male and female mice, these symptoms were not observed in the animals in the control group. During the exposure, the animals (both sexes) in the treatment group were calm. There was neither sedation nor alopecia in both the control and treatment groups (Table 1). The data on body weight, after sub-acute treatment with Bakhour smoke showed no changes in the post-treatment body weight in both the sexes, as compared to the body weight in the pre-treatment group (Table 2). Our observation on the average organ weight also showed no changes in the organ weight of heart liver, kidney, spleen, testis and uterus, however; there was a significant reduction ($P<0.05$; $P<0.001$) in the mean weight of lungs of male and female, as compared to the control (Table 3). The smoke was found to have no effect on rate of mortality. The sub-acute treatment with Bakhour smoke failed to affect the frequency of micronucleated-polychromatic erythrocytes (PCE) in both male and female mice A comparison of PCE and the normochromocytic erythrocytes (NCE) showed that the changes in the PCE/NCE ratio were insignificant (Table 4).

Table 1: Effect of Bakhour smoke exposure on general symptoms of toxicity observed after sub-acute treatment in male and female Swiss albino mice.

	Control		Bakhour (100 g) smoke Exposure.	
Toxicity symptoms	Male	Female	Male	Female
Autonomic Responses				
Respiration	-	-	++	++
Hypothermia	-	-	-	-
Hyperthermia	-	-	-	-
Heart rate	-	-	-	-
Pilo erection	-	-	+	+
Salivation	-	-	-	-
Micturation	-	-	-	-
Defecation	-	-	-	-
Writhing	-	-	-	-
Motor Activity	-	-	-	-
Staggering	-	-	-	-
Reflex impairment	-	-	-	-
Cns Excitation				
Straub tail	-	-	-	-
Tremors	-	-	-	-
Convulsions	-	-	-	-
Twitches	-	-	-	-
Aggression	-	-	-	-
Excitation	-	-	-	-
Itching	-	-	+	+
Waltzing movements	-	-	-	-
Muscle tone	-	-	-	-
Ocular	-	-	-	-
Dermal	-	-	-	-
Symptoms Other than above				
Sedation	-	-	-	-
Calmness	-	-	+	+
Alopecia	-	-	-	-

10 mice were used in each group. - = Absent + = Mild; ++ = Moderate,

Table 2: Effect of Bakhour on body weight after sub-acute treatment in male and female Swiss albino mice.

		Body weight (Mean ± S.E.)			
		Male		Female	
Sl. No.	Treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
1	Control	26.55±1.41	28.30±1.18	25.91±1.12	27.85±1.79
2	Bakhour (100 g.) Smoke Exposure	25.93±1.06	27.15±1.81	26.75±1.43	27.98±1.63

Ten mice were used in each group; Post treatment group was compared to Pre-treatmentGroup in each sex.

Table 3: Effect of Bakhour on average organ weight after Sub-acute treatment in Swiss albino mice.

	Mean weight of the organ per 100 gm. body weight ± S.E											
	Heart		Lungs		Liver		Kidney		Spleen			
Treatment	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Testis	Uterus
Control	0.55±0.01	0.56±0.05	1.01±0.01	1.02±0.06	7.05±0.42	6.17±0.26	0.79±0.03	0.79±0.02	0.64±0.04	0.54±0.03	0.48±0.08	0.45±0.06
Bakhour (100 g.) Smoke Exposure	0.55±0.01	0.51±0.03	0.77±0.03*	0.70±0.01**	6.99±0.15	5.41±0.16	0.74±0.05	0.74±0.02	0.73±0.05	0.49±0.02	0.38±0.03	0.56±0.04

Five mice were used in each group Groups 2, was compared to group 1. *P<0.05; **P<0.001 (One-way ANOVA followed by Tukey-Kramer multiple comparison test was used)

Table 4: Effect of Bakhour on the frequency of micronuclei and the ratio of polychromatic to normochromatic Erythrocytes in femoral cells of Swiss albino mice after acute treatment.

		Polychromatic erythrocytes (PCE) screened		Micro-nucleated Polychromatic erythrocytes (%) (Mean ± S.E.)		Normochromatic erythrocytes (NCE) screened		PCE/NCE ratio (Mean ± S.E.)	
Serial	Treatment	Male	Female	Male	Female	Male	Female	Male	Female
1.	Control	5150	5150	0.29±0.03	0.28±0.05	5042	4824	1.02±0.08	1.07±0.09
2.	Bakhour (100 g.) Smoke Exposure	5525	5450	0.30±0.04	0.29±0.02	5260	5550	1.05±0.05	0.98±0.06

Five mice were used in each group

DISCUSSION

The results on general toxicity of the exposure to Bakhour smoke showed no significant toxicity in any of the parameters on autonomic responses, motor activity, CNS excitation and other symptoms of sedation, calmness and alopecia, body weight, organ weight, mortality and genetic toxicity except mild calmness, itching and pilo erection and moderate effect on respiration and significant reduction of weight of lungs in both male and male and female mice. These results clearly demonstrate lack of toxic potentials in the short term exposure to Bakhour smoke. The effects on respiration, pilo erection, itching are obvious upon exposure to smoke which might have caused little reaction to the suffocation, which is reflected in reduced weight of lungs observed in the same study. Nonetheless, no correlation was seemingly detected between exposure to incense burning and respiratory symptoms (chronic cough, chronic sputum, chronic bronchitis, runny nose, wheezing, asthma, allergic rhinitis, or pneumonia) in an extensive study conducted by several Asian Cancer Research Centers [19].

In the absence of a similar study on Bakhour, it is difficult to interpret the results, nevertheless; based on the synonymy in the smoke of Bakhour and incense the comparison of these results is extended to literature reports on incense smoke. The contents of the incense smoke (CO, NO2, SO2, volatile organic compounds, PAHs and toxic metals) [6] are widely reported to be toxic, mutagenic and carcinogenic. Lin and Wang, [20] reported burning of incense sticks accumulated carcinogens, including PAHs and benzopyrene. The authors analyzed that gaseous aliphatic aldehydes in incense smoke are carcinogenic and mutagenic. A survey on risk factors for lung cancer observed an inverse association between incense burning and adenocarcinoma of the lung [21]. Chen and Lee [22] analyzed incense smoke condensate (ISC) using the Ames test in S. Typhimurium strains with different mutagenic specificity and level of metabolic enzyme, the SOS chromotest in *E. coli* PQ37 and sister chromatid exchange assay in Chinese hamster ovary cells and obtained a positive response in TA98, but not in TA 100. The authors suggested partly the nitroarenes to be responsible for the observed mutagenicity. Kim *et al*. [23] showed the highest emission factor of 11 genotoxic PAH from kerosene smoke emanated from domestic stoves. A population-based case-referent study has been conducted to examine the effect of incense smoke exposure on lung cancer risk among Chinese males and explored the joint effect of cigarette smoking and exposure to residential radon. This study suggested that incense burning influenced the toxic impact of cigarette smoking and exposure to radon [24].

Although, a large number of papers are published on the toxicity of incense smoke and its constituents, little is known about the toxicity and/or genotoxicity or carcinogenicity of the smoke from Bakhour, except the work on respiratory toxicity. Alarifi *et al.* [14] found ultra structural changes in the Alveolar pneumocytes of Bakhour-exposed animals. These changes involved the cell organelles and surfactant material of type II cells. Hyperplasia of alveolar cells was a feature in the affected lung tissue. Neutrophils accompanied with degenerative and necrotic changes were found infiltrating pulmonary alveoli and of the alveolar cells. There was a thick deposition of collagen fibrils in the alveolar walls. Al-Rawas *et al*. [16] also showed that bakhour burning to adversely affect respiratory health. However, literature is devoid of any report on the composition, mutagenicity and/or carcinogenicity of smoke emanating from Bakhour. Furthermore, there is no report on the synonymy between the contents of Bakhour smoke and incense smoke, especially, the PAHs content. Nevertheless, a detailed study on the composition of Bakhour smoke and toxicity and genotoxicity of its individual constituents is suggested.

CONCLUSION

The study on general symptoms of toxicity and genetic toxicity conducted in this preliminary study showed that Bakhour smoke is non- toxic. The source of manufacture of Bakhour and/or any incense is significant in considering the toxicity due to smoke. There is limited research on Bakhour used in the Arabian countries, hence; it is imperative to explore the identity of the fragrant smoke emanating from different types of incense and bakhour. The composition and individual constituents of Bakhour smoke and its source may be identified and their toxicity explored. The mutagenicity tests that cannot be compared to an in vivo situation in human beings may not be used to determine the genetic toxicity.

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