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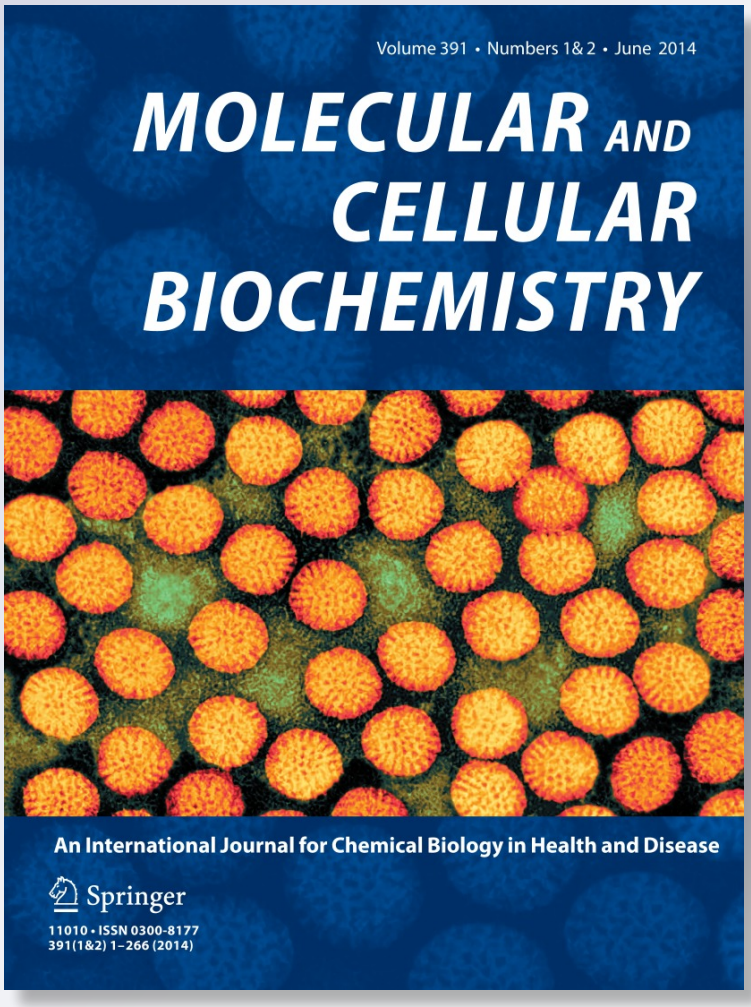

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Induction of CYP1A1, CYP1A2, CYP1B1, increased oxidative stress and inflammation in the lung and liver tissues of rats exposed to incense smoke

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Abstract Incense smoke is increasingly being recognized as a potential environmental contaminant and is linked to malignant and non-malignant respiratory diseases. The detoxification of environmental contaminants including polycyclic aromatic hydrocarbons (PAHs) involves the induction of cytochrome P-450 family enzymes (CYPs) by PAHs. However, the detoxification of PAHs also results in the generation of reactive and unstable intermediary metabolites which are implicated in the oxidative stress, DNA damage, and inflammation. It is unclear whether CYPs are similarly induced by incense smoke, which incidentally contains substantial amounts of PAHs. Here, we examined the impact of long-term incense smoke exposure on the induction of CYPs in male Wister Albino rats. Incense smoke exposure significantly induced the expression of CYP1A1, CYP1A2, and CYP1B1 mRNAs in both lung and liver tissues. The extent of CYP1A1 and CYP1B1 induction was significantly higher in the liver compared to that in the lung, while that of CYP1A2 was greater in the lung than in liver. Incense smoke exposure also increased malondialdehyde and reduced glutathione levels in lung and liver tissues, and the catalase activity in the liver tissues to significant levels. Furthermore incense smoke exposure led to a marked increase in TNF-α and IL-4 levels. The data demonstrate for the first time the capacity of incense smoke to induce CYP1 family enzymes in the target and non-target tissues. Induction of CYPs increased oxidative stress and inflammation appear to be intimately linked to promote the carcinogenesis and health complications in people chronically exposed to incense smoke.

Keywords Incense · Cytochrome p-450 · Lung · Liver · Inflammation · Oxidative stress

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Introduction

Incense burning is an integral part of daily lives in large parts of Asia. Besides its use in the places of worship, a vast majority of the populations across South-East Asia and Middle Eastern countries burn incense in homes on a daily basis as a source of fragrance [1, 2]. The incense used in Asian countries is made from plant materials mixed with essential oils and forms a combustible mixture which releases fragrant smoke upon burning. The most common types of incense used in the Gulf countries are referred to as bakhour and oudh. A wide variety of substances are used to produce bakhour, including sandal wood tree resin, agar wood, essential oils, and perfumes, whereas oudh is a homogenously made agar wood from Aquilaria agallocha, which develop an aromatic smell due to fungal infection [3, 4]. The incense smoke characteristics vary worldwide

mainly due to variations in the incense composition. Typically, the incense smoke contains particulate matter of varying particle size, gases such as CO, CO_2, NO_2, and SO_2, volatile organic compounds including benzene, toluene, and xylenes, as well as aldehydes and PAHs [1, 5, 6].

Due to the restricted ventilation and the amount of time people spent indoors, the likelihood and intensity of exposure to environmental contaminants such as incense and cigarette smoke are expected to be greater in indoors than outdoors. Incense smoke exposure is increasingly being reported to be detrimental to human health. Although incense smoke contains multitude of toxicants, the particulate matter, PAHs, carbonyls, and benzene are suggested to be the major components exerting negative effects of incense smoke on human health [7–9]. Since inhalation is a mode of exposure, a large number of studies have focused on the effects of incense smoke on respiratory tract carcinomas and other airway diseases including asthma and chronic obstructive pulmonary disease (COPD) [10–13]. Besides, incense smoke exposure has also been linked to allergic contact dermatitis [14]. We have earlier shown that long-term Arabian incense smoke exposure of rats contributes to insulin resistance, dyslipidemia, and increased adipocytokine production [4]. Recently, we found that incense exposure alters spermatogenesis and sperm parameters in rats exposed to Arabian incense smoke [15]. At cellular level, several studies have described the incense smoke to induce genotoxicity, increase cellular oxidative stress and inflammation, and these events are suggested to be responsible for the reported negative effects of incense smoke on cancer risk and respiratory complications [16–21].

The CYP dependent monooxygenase system is the primary route through which the environmental contaminants including PAHs are metabolized and detoxified in mammals [22, 23]. Members of the CYP family most active in the metabolism of PAHs and other chemical carcinogens include CYP1A1, CYP1A2, CYP1B1, and CYP2E1 [23–25]. Metabolism of PAHs involves the induction of CYP enzymes by PAHs per se and the subsequent detoxification of toxic contaminants. However, the metabolic process of PAHs also results in the production of bioactive and unstable intermediary metabolites that are capable of generating oxidative stress, inflammation, and inducing DNA damage [26–29]. Thus, the enhanced expression of CYP enzymes is not only critical in the detoxification of environmental contaminants but also poses the risk of increased generation of carcinogenic intermediary metabolites. Considering that incense smoke contains substantial amount of PAHs, we suspected that CYP1A1, CYP1A2, and CYP1B1 are similarly induced by the incense smoke, and the induction of these CYPs contributes to oxidative stress and increased inflammation. Toward this end, we examined the expression of CYP1A1, CYP1A2, and CYP1B1 as well as oxidative stress and inflammatory markers in the lung and liver tissues of rats after exposing to Arabian incense smoke.

Materials & methods

Animals and incense

Male Wistar albino rats (*Rattus norvegicus*) aged 7–8 weeks, weighing 200–210 g, were obtained from the Animal Care Center, College of Pharmacy, King Saud University; Riyadh. The Ethics Committee of the Experimental Animal Care Center approved the study. Animals were housed in a temperature-controlled facility on a 12 h light/dark cycle and had access to water and normal chow diet ad libitum. In the present study, we selected the two most commonly used incense in the Middle Eastern region namely bakhour and oudh.

Exposure to incense smoke

After two week acclimatization period, rats were randomly divided into three groups namely control, bakhour, and oudh with each group containing 24 animals. Each group of rats was housed separately from the other to avoid the cross exposure of incense smoke. Rats from bakhour and oudh groups were subjected to whole body exposure of incense smoke emanated from the burning of 4 g of respective incense for about 60 min daily. Rats were exposed to incense smoke in an inhalation chamber with dimensions of 72 cm by length, 43 cm by width, and 35 cm by height resulting in a volume of 108 L. The ratio of chamber volume to the mean body weight of rats was in proportionate to that of the ratio of standard body weight of individuals to the standard living room size. In addition, the amount of incense was scaled down in accordance with the mean of the body weights of the rats viz. a viz. standard body weight of individuals, simulating the indoor incense burning. Rats from the control group were maintained in fresh air. Eight rats from each group were killed after 30, 60, and 90 days from the commencement of the incense smoke exposure. Lung and liver tissues were excised and snap frozen in liquid nitrogen and stored at $-80\ ^{\circ}C$ until analyzed. Since the lung is the target tissue as the inhalation is the rout of incense smoke exposure and the liver is the principal site of the bioactivation of the environmental contaminants, these tissues were chosen to study the effect of incense smoke.

Table 1 Primers to amplify the target sequences

Target gene	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	Product size
CYP1A1	CCA TGA CCA GGA ACT ATG GG	TCT GGT GAG CAT CCA GGA CA	341
CYP1A2	CGCATTGGCTCCACACCCGTG	ACCGATTCCACCACCTGGTTGACT	353
CYP1B1	ACC GCA ACT TCA GCA ACT TC	GTG TTG GCA GTG GTG GCA TG	427
GAPDH	TTGGCCGTATCGGACGCCTG	AGCGGAAGGGGCGGAGATGA	347

CYP1A1 cytochrome P-450 1A1, 1A2, 1B1; *GAPDH* glyceraldehyde phosphate dehydrogenate

Real-time PCR

In this study, we evaluated the effect of incense smoke on CYP1A1, CYP1A2, CYP1B1, and CYP2E1 because of their active participation in the metabolism and bioactivation of xenobiotics [22–25]. In addition, CYP1A1, CYP1A2, CYP1B1 genes contain the xenobiotic response elements, resulting in their induction by aryl hydrocarbon receptors which are activated by binding of xenobiotic ligands such as PAHs [30, 31]. The relative expression of CYP1A1, CYP1A2, and CYP1B1 genes in control and incense smoke exposed rats was evaluated by real-time PCR. Total RNA was isolated from the lung and liver tissues using RNeasy mini kit (Qiagen, CA, US), and 1 μg of total RNA was reverse transcribed to cDNA using QuantiTect Reverse Transcription Kit (Qiagen, CA, US). Real-time PCR was performed on CFX96 Real-Time PCR system (Bio-Rad Laboratories, Hercules, CA,USA) in a 96 well plate using SYBR green master mix (Kappa Bioscience, MA, US), 10 μmol each of forward and reverse primers to amplify the target genes (Table 1) and cDNA equivalent to 100 ng of total RNA. The PCR was carried out with an initial cycle of 94 °C for 5 min followed by 40 cycles, each consisting of 94 °C, 15 s and 58 °C, 1 min. The GAPDH gene was amplified as an internal control. Each sample was amplified in triplicates. The data were analyzed by ΔΔCt method.

Measurement of MDA

For the determination of malondialdehyde (MDA), the liver and lung tissues were homogenized in 100 mmol KH_2PO_4 buffer containing 1 mmol EDTA (pH 7.4). Tissue homogenates were centrifuged at 12,000 × g for 30 min at 4 °C. The MDA content in the clear supernatants was determined according to Draper and Hadley [32]. Briefly, 0.5 ml of liver and lung tissue extract supernatants was mixed with 1 ml of trichloroacetic acid solution and centrifuged at 2,500 g for 10 min. The clear supernatant [0.5 ml] was mixed with 1 ml solution containing 0.67 % thiobarbituric acid (TBA) and incubated for 15 min at 90 °C. The absorbance of the solution was recorded at 535 nm, and the concentration of MDA was calculated and expressed as nmol/mg protein.

Measurement of catalase activity

The catalase activity was assayed according to the method by Aebi [33] in a final reaction volume of 3 ml contained 0.05 M Trisbuffer, 5 mM EDTA (pH 7.0), and 10 mM H_2O_2 in 0.1 M potassium phosphate buffer, (pH 7.4). A volume of 50 μl of the supernatant of tissue homogenate was added to the above reaction mixture. The rate of change in absorbance per min at 240 nm was recorded. Catalase activity was expressed in terms of μmoles H_2O_2 consumed/min per milligram of protein.

Measurement of glutathione

The measurement of glutathione (GSH) in the lung and liver tissues was carried out according to the procedure reported by Owen [34]. Briefly, 100 μl of clear supernatant of lung or liver tissue homogenate was mixed with 800 μl of 0.3 mM reduced NADPH, 100 μl of 6 mM 5,5-dithiobis-2-nitrobenzoic acid (DTNB), and 10 μl of 50 units/ml GSH reductase. All these reagents were prepared freshly in a phosphate buffer at pH 7.5. The absorbance was measured over a period of 120 s at 412 nm at 30 °C. The GSH level was determined by comparing the rate of change in absorbance of the test solution with that of standard GSH.

Measurement of TNF-α and IL-4

The concentrations of TNF-α and IL-4 in the lung and liver tissues were determined by competitive ELISA as described by the manufacturer (My Biosource, CA, US). Briefly, the tissue homogenate and the TNF-α-HRP conjugate or IL-4-HRP conjugate were added to the plate precoated with the anti-TNF-α or IL-4 antibodies, respectively. Plates were incubated for 1 h, washed and incubated with the HRP substrate, 3, 3′, 5, 5′-Tetramethylbenzidine. The absorbance was measured at 450 nm using microplate reader. The concentrations of TNF-α and IL-4 were calculated from the standard curve generated by similarly processing the TNF-α and IL-4 standards.

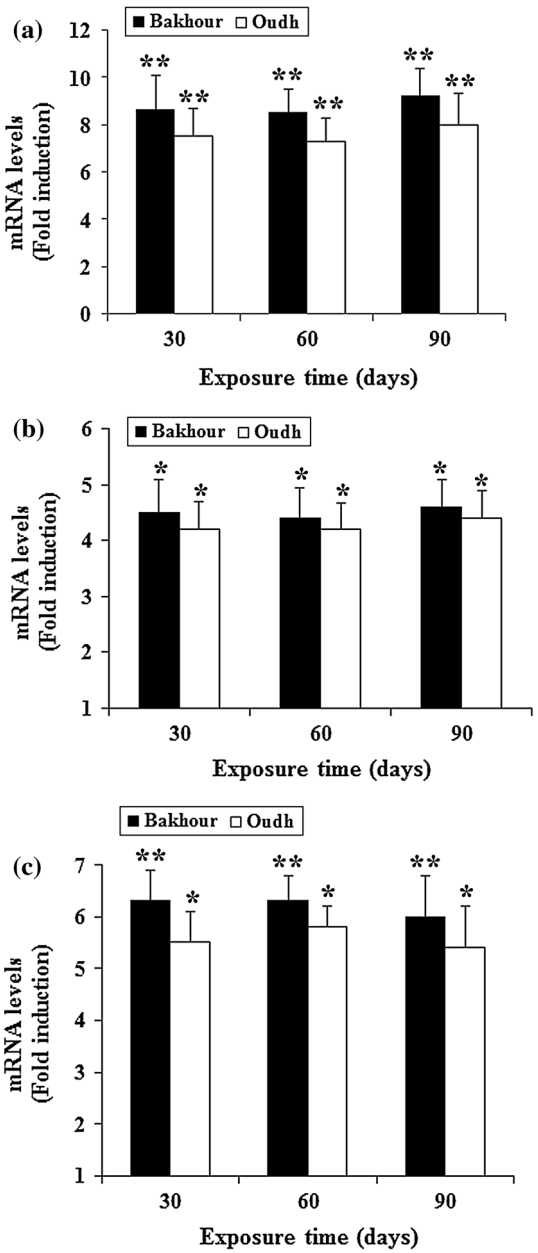

Fig. 1 Fold induction of CYP1A1, CYP1A2, and CYP1B1 mRNAs in the lung tissues of rats exposed to incense smoke above the respective mRNAs in the unexposed control rats. Rats were exposed to smoke released by the burning of 4 g each of bakhour or oudh for 30, 60, or 90 days. Total RNA was extracted from the lung tissues of exposed and unexposed control rats, and the mRNA levels of respective genes were measured by Real-Time PCR. The results represent the mean ± SD ($N = 8$). *$p < 0.05$ and **$p < 0.001$

Statistical analysis

The SPSS software was used for statistical analysis. The data were represented by mean ± standard deviation. All non-Gaussian variables were appropriately transformed, prior to the parametric analysis. Analysis of variance (ANOVA) was done comparing different groups, followed by Dunnett's multiple comparison post-hoc test. The level of significance was given at $p < 0.05$.

Results

Induction of CYP1 family genes in the lung tissue

Fold induction of CYP1A1, CYP1A2, and CYP1B1 in the lung tissues of rats exposed to bakhour and oudh is presented in Fig 1. The mean fold induction of CYP1A1 in the lung tissue homogenates of rats exposed to bakhour for 30 days was 8.6 ($p < 0.001$) above the time matched CYP1A1 expression in the unexposed control rats. Compared to time matched CYP1A1 expression in unexposed control rats, the mean fold induction of CYP1A1 remained significantly higher even when the rats were exposed to bakhour for 60 (8.5 fold, $p < 0.001$) or 90 days (9.2 fold, $p < 0.001$). The mean fold induction of CYP1A1 was found to be 7.5 ($p < 0.001$) in the lung tissues of rats exposed to oudh for 30 days compared to time matched expression in the unexposed rats. The effect of oudh on the induction of CYP1A1 in rats exposed for 60 (7.3 fold, $p < 0.001$) or 90 (8 fold, $p < 0.001$) days was comparable to that found at 30 day exposure, and the induction was significantly different compared to time matched expression in unexposed control rats. Thus, the CYP1A1 was induced maximally after 30 days of exposure, and no further change in the extent of induction was noticed after 60 or 90 days of exposure. There was no statistical difference in the induction of CYP1A1 in lung tissues of rats exposed to bakhour or oudh at any given time.

The mean fold induction of CYP1A2 in the lung tissues of rats was found to be 4.5 ($p < 0.05$), 4.4 ($p < 0.05$), and 4.6 ($p < 0.05$) following exposure to bakhour for 30, 60, or 90 days, respectively, compared to time matched expression in the unexposed control rats. The exposure of rats to oudh for 30, 60, or 90 days significantly induced the expression of CYP1A2 by 4.2 ($p < 0.05$), 3.9 ($p < 0.050$), and 4.4 ($p < 0.05$) folds, respectively. Consistent with the CYP1A1 induction, CYP1A2 was induced maximally after 30 days of exposure, and no further change in the extent of induction was noticed even after 60 or 90 days of exposure No statistically significant difference was found between bakhour and oudh with regard to the extent of CYP1A2 induction at any given time point.

Exposure of rats to bakhour for 30, 60, or 90 days also upregulated the CYP1B1 expression by 6.3 ($p < 0.0010$), 6.3 ($p < 0.001$), and 6 ($p < 0.001$) folds, respectively, in the lung tissues compared to time matched expression in the control rats. The mean fold induction of CYP1B1 in the lung tissues of rats exposed to oudh was 5.5 ($p < 0.05$), 5.8

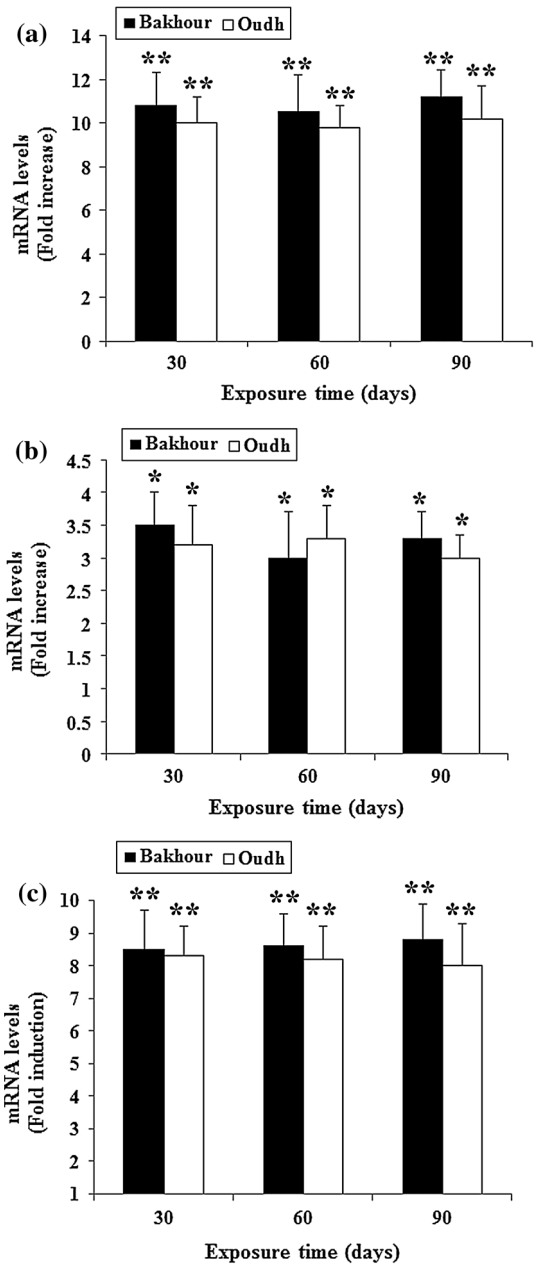

Fig. 2 Fold inductions of CYP1A1, CYP1A2, and CYP1B1 mRNAs in the liver tissues of rats exposed to incense smoke above the respective mRNAs in the unexposed control rats. Rats were exposed to smoke released by the burning of 4 g each of bakhour or oudh for 30, 60, or 90 days. Total RNA was extracted from the liver tissues of exposed and unexposed control rats, and the mRNA levels of respective genes were measured by Real-Time PCR. The data are the mean ± SD ($N = 8$). *$p < 0.05$ and **$p < 0.001$

($p < 0.05$), and 5.4 ($p < 0.05$) folds following exposure for 30, 60, and 90 days, respectively. The induction of CYP1B1 by oudh at the three time points was identical to that by bakhour.

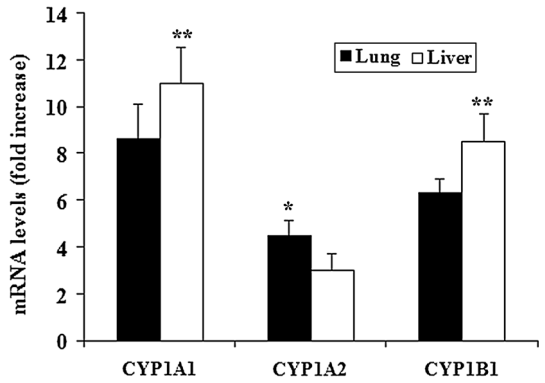

Fig. 3 Differential induction of CYP1A1, CYP1A2, and CYP1B1 mRNAs in the lung and liver tissues of rats exposed to incense smoke. Rats were exposed to smoke released by the burning of 4 g of bakhour daily for 30 days. Total RNA was extracted from the lung and liver tissues, and the mRNA levels of respective genes were measured by Real-Time PCR. The data represent the mean ± SD ($N = 8$). *$p < 0.05$ and **$p < 0.01$

Induction of CYP1 family genes in the liver tissue

The mean fold induction of CYP1A1, CYP1A2, and CYP1B1 in the liver tissues of rats exposed to bakhour and oudh is presented in Fig 2. Bakhour exposure of rats for 30, 60, and 90 days resulted in 11 ($p < 0.001$), 10.5 ($p < 0.001$), and 11.2 ($p < 0.001$) fold induction of CYP1A1, respectively, in the liver tissues. In comparison of control, oudh exposure significantly induced CYP1A1 after 30 (10 folds, $p < 0.001$), 60 (9.8 folds, $p < 0.001$), and 90 (10.2 folds, $p < 0.001$) days. Both bakhour and oudh could be able to maximally induce the CYP1A1 after 30 days of exposure as no further increase in the mRNA levels was noticed following the exposure for 60 or 90 days.

The CYP1A2 was induced by 3.0 fold in the liver in response to bakhour exposure of rats for 30 days, and the magnitude of induction was sustained with the continued exposure for 60 (3 folds, $p < 0.050$) or 90 days (3.3 fold, $p < 0.05$). The exposure of rats by oudh for 30, 60, or 90 days had a 3.2 ($p < 0.050$), 3.3 ($p < 0.05$), and 3.0 ($p < 0.05$) fold induction of CYP1A2. Thus, the induction of CYP1A2 by bakhour and oudh was comparable.

Matched to control rats, bakhour exposed rats had 8.5 ($p < 0.001$), 8.6 ($p < 0.001$), and 8.8 ($p < 0.001$) fold induction of CYP1B1 after 30, 60, or 90 days of exposure in the liver tissue, while exposure of rats for the identical time points by oudh led to 8.3 ($p < 0.001$), 8.2 ($p < 0.001$), and 8 ($p < 0.001$) folds increase in the CYP1B1 expression. There was no statistically significant variation in the expression of CYP1B1 by bakhour and oudh.

Table 2 Oxidative stress markers in the lung tissues of rats exposed to bakhour or oudh smoke

Markers	30 days			60 days			90 days		
	Control ($N = 8$)	Bakhour ($N = 8$)	Oudh ($N = 8$)	Control ($N = 8$)	Bakhour ($N = 8$)	Oudh ($N = 8$)	Control ($N = 8$)	Bakhour ($N = 8$)	Oudh ($N = 8$)
MDA (nmol/mg)	2.28 ± 0.51	4.51 ± 0.64*	3.46 ± 0.55*	2.15 ± 0.41	4.61 ± 0.67*	3.48 ± 0.53*	1.98 ± 0.32	3.55 ± 0.45*	3.45 ± 0.48*
GSH (nmol/mg)	45.4 ± 5.12	82.6 ± 6.31*	66.7 ± 5.94*	42.5 ± 5.80	71.1 ± 6.45*	67.3 ± 5.87*	40.32 ± 6.21	69.4 ± 6.6*	70.6 ± 6.32*
Catalase (pmol/min mg)	1.42 ± 0.18	1.54 ± 0.16	1.48 ± 0.21	1.36 ± 0.22	1.41 ± 0.26	1.55 ± 0.15	1.51 ± 0.26	1.45 ± 0.23	1.36 ± 0.19

GSH-reduced GSH

MDA malondihaldehyde

*$p < 0.001$

Tissue specific expression of CYP1 genes

Comparison of CYP1A1, CYP1A2, and CYP1B1 induction between lung and liver of rats exposed to bakhour was shown in Fig 3. Significant tissue specific differences were found in the inductions of CYP1A1, CYP1A2, and CYP1B1 in the rats exposed to bakhour for 30 days. A 2.4 ($p < 0.01$) and 2.2 ($p < 0.01$) folds higher induction of CYP1A1 and CYP1B1 were found in the liver compared to those in the lung. Contrarily, CYP1A2 showed a 1.5 fold ($p < 0.050$) higher induction in the lung compared to that in the liver. Similar significant differences were found in the extent of CYP1A1, CYP1A2, and CYP1B1 inductions between lung and liver tissues at 60 and 90 days exposure durations in response to bakhour and oudh (data not shown).

Oxidative stress markers in the lung and liver

The levels of oxidative stress markers including MDA, GSH, and catalase activity in the lung and liver tissues are presented in Table 2 and 3, respectively. The MDA and GSH levels were significantly increased in the lung tissues of rats exposed to bakhour and oudh for 30 days in relation to those in the unexposed control rats. Compared to time matched control, the lung MDA and GSH levels remained significantly elevated when the exposer with bakhour or oudh was continued for 60 or 90 days. No statistical difference was found in the lung catalase activity in the bakhour or oudh exposed rats compared to unexposed control rats. Similarly, the MDA and GSH levels were significantly higher in the liver tissues of rats exposed to bakhour or oudh for 30, 60, or 90 days compared to time matched controls. Interestingly, catalase activity significantly altered in the liver tissues of rats exposed to both bakhour and oudh after 60 day exposure compared to control.

Inflammatory markers in the lung

The concentrations of inflammatory markers including TNF-α and IL-4 in the lung tissues are presented in Fig 4. Compared to control, TNF-α levels were significantly increased in the lung tissues of rats exposed to bakhour for 30 (45.2 ± 10.0 vs. 80.4 ± 7.2 pg/g, $p < 0.001$), 60 (42.3 ± 7.4 vs. 82.4 ± 11.0 pg/g, $p < 0.001$), or 90 (40.7 ± 6.5 vs. 78.3 ± 8.1 pg/g, $p < 0.001$) days. A similar significant increase in TNF-α levels in the lung tissues was noticed after the rats were exposed to oudh for 30 (45.2 ± 10.0 vs. 75.6 ± 8.2 pg/g, $p < 0.001$), 60 (42.3 ± 7.4 vs. 70.5 ± 8.6 pg/g, $p < 0.001$), or 90 (40.7 ± 6.5 vs. 81.3 ± , 9.8 pg/g, $p < 0.001$) days compared to that in the control rats. Compared to control, bakhour significantly increased the IL-4 levels in the lung following exposure for 30 (15.3 ± 2.3 vs. 26.9 ± 4.2 pg/g, $p < 0.001$), 60 (16.2 ± 2.3 vs. 26.1 ± 5.2 pg/g, $p < 0.001$), or 90 (16.0 ± 2.3 vs. 25.6 ± 3.9 pg/g, $p < 0.001$) days. Matched to control, IL-4 levels in the lung tissues were also significantly elevated in response to oudh exposure for 30 (15.3 ± 2.3 vs. 23.5 ± 3.3 pg/g, $p < 0.001$), 60 (16.2 ± 2.0 vs. 24.2 ± 3.9 pg/g, $p < 0.001$), or 90 (15.9 ± 1.9 vs. 22.6 ± 3.5 pg/g, $p < 0.001$) days.

Inflammatory markers in the liver

Both bakhour and oudh had significant positive effects on liver TNF-α levels (Fig 5). Compared to those in the control rats, the liver TNF-α levels in bakhour exposed rats significantly differed after exposure for 30 (21.3 ± 3.2 vs. 32.4 ± 4.2 pg/g, $p < 0.001$), 60 (22.4 ± 2.8 vs. 34.4 ± 4.2 pg/g, $p < 0.001$), or 90 days (20.6 ± 3.1 vs. 33.6 ± 5.1 pg/g, $p < 0.001$). A similar increase in TNF-α levels was noted in oudh exposed rats in comparison with control after 30 (21.3 ± 3.2 vs. 30.8 ± 4.5 pg/g, $p < 0.001$), 60 (22.4 ± 2.8 vs. 31.7 ± 3.8 pg/g, $p < 0.001$), or 90 (20.6 ± 3.1 vs. 29.8 ± 3.5 pg/g, $p < 0.001$) days exposure. Liver IL-4 levels significantly differed between control rats and rats exposed to bakhour for 30 (15.9 ± 3.0 vs. 26.4 ± 3.2 pg/g, $p < 0.001$), 60 (17.6 ± 2.3 vs. 30.2 ± 3.8 pg/g, $p < 0.001$), or 90 (16.7 ± 2.5 vs. 29.4 ± 3.2 pg/g, $p < 0.001$) days. In relation to unexposed rats, oudh exposed rats exhibited a significant increase in the liver IL-4 levels after 30 (15.9 ± 3.0 vs. 27.8 ± 4.2 pg/g, $p < 0.001$), 60

Table 3 Oxidative stress markers in the liver tissues of rats exposed to bakhour or oudh smoke

Markers	30 days			60 days			90 days		
	Control ($N = 8$)	Bakhour ($N = 8$)	Oudh ($N = 8$)	Control ($N = 8$)	Bakhour ($N = 8$)	Oudh ($N = 8$)	Control ($N = 8$)	Bakhour ($N = 8$)	Oudh ($N = 8$)
MDA (nmol/mg)	1.18 ± 0.38	2.52 ± 0.41**	2.43 ± 0.45**	1.15 ± 0.28	2.61 ± 0.40**	2.48 ± 0.44**	0.98 ± 0.031	2.55 ± 0.028**	2.12 ± 0.037**
GSH (nmol/mg)	35.4 ± 3.31	68.6 ± 5.2**	55.3 ± 6.1**	34.7 ± 4.2	71.1 ± 6.8**	70.3 ± 6.5**	36.3 ± 4.1	72.4 ± 6.2**	69.6 ± 7.0**
Catalase (pmol/min/mg)	0.42 ± 0.040	0.39 ± 0.043	0.45 ± 0.054	0.36 ± 0.036	0.41 ± 0.052*	0.43 ± 0.041*	0.44 ± 0.038	0.45 ± 0.043	0.42 ± 0.039

GSH-reduced GSH

MDA malondialdehyde

*$p < 0.05$; **$p < 0.01$

(17.6 ± 2.3 vs. 26.8 ± 3.8 pg/g, $p < 0.001$), or 90 (16.7 ± 2.5 vs. 28.1 ± 3.7 pg/g, $p < 0.001$) days of exposure.

Discussion

Incense burning is widely practiced in majority of South East Asian countries and in Middle East. Incense is burned in households and religious places as a tradition or a source of fragrance. Incense burning releases considerable amounts of harmful gases, particulate matter, and other substances including CO, CO_2, NO_2, and SO_2, benzene, aldehydes, and PAHs into the environment. The distribution and density of these environmental contaminants are reported to be far higher in the vicinity of places where incense is burned, making the people more vulnerable to the inhalation of these agents [2, 17, 18]. A number of studies have identified the incense smoke to be carcinogenic in nature which is attributed to its components particularly the benzene, carbonyls, and PAHs due to their ability to induce DNA damage and reduce the DNA repair capacity [7, 8, 17, 18]. Moreover, incense smoke is shown to increase ROS production, oxidative stress, and the production of inflammatory mediators [19, 20, 35, 36]. Despite the well-described carcinogenic nature of incense smoke, the underlying mechanism associated with its carcinogenicity is not fully understood. In the present study, we examined the effects of chronic incense smoke exposure on the expression of CYP1A1, CYP1A2, CYP1B1, oxidative stress, and inflammation in lung and liver tissues in rats.

The environmental contaminants such as PAHs are metabolized by cytochrome P-450 enzymes, particularly the CYP1A1, CYP1A2, CYP1B1, and CYP2E1 [22–25]. However, during the course of metabolism of PAHs a number of bioactive and unstable intermediary products are formed which are more toxic than the parent compound and can elicit the oxidative stress and DNA damage [37]. In fact, in vivo and in vitro studies have shown a significant correlation between increased CYP1A1, CYP1A2, and CYP1B1 expressions and augmented DNA adduct formation following the exposure to PAHs [26–29]. A number of reports have demonstrated the cigarette smoke, a well-established source of multitude of toxic agents including carcinogenic PAHs, as well as smoke from other combustion sources to upregulate the expression of CYPs, increase oxidative stress and induce genotoxicity in lung tissues or bronchial epithelial cells [38–41]. Cigarette smoke has also been reported to upregulate CYPs and to induce DNA strand breaks in the liver [42, 43]. Collectively, these studies indicate that cellular events, triggered following the induction of CYPs, are suggested to enhance the risk of lung and liver malignancies. In this study, we showed for the first time the potential of incense smoke to

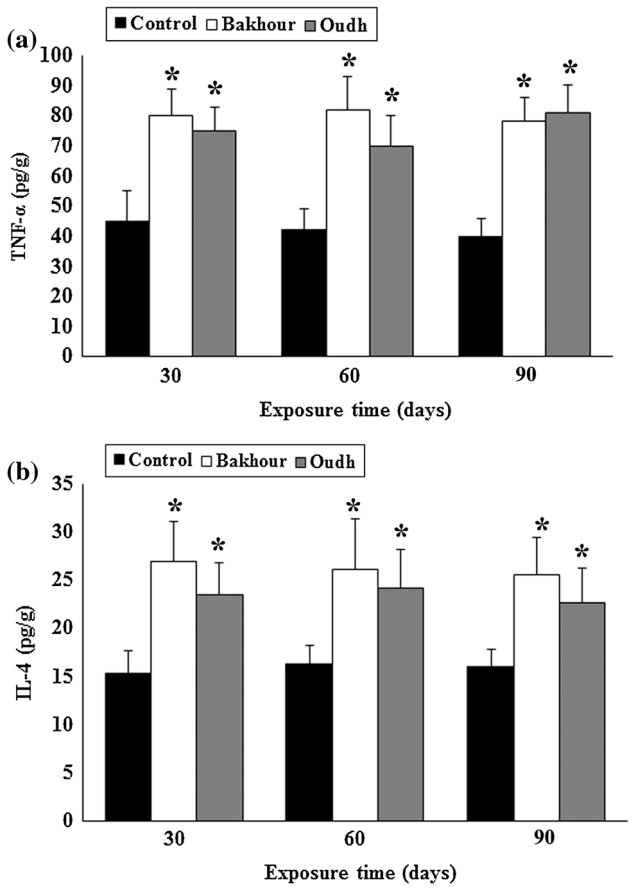

Fig. 4 TNF-α and IL-4 levels in the lung tissue of control and incense smoke exposed rats. Rats were exposed to smoke released by the burning of 4 g each of bakhour or oudh daily for 30, 60, or 90 days. The TNF-α and IL-4 levels in the lung tissue homogenates of exposed or unexposed control rats were measured by ELISA-based method. The data represent the mean ± SD ($N = 8$), *$p < 0.001$

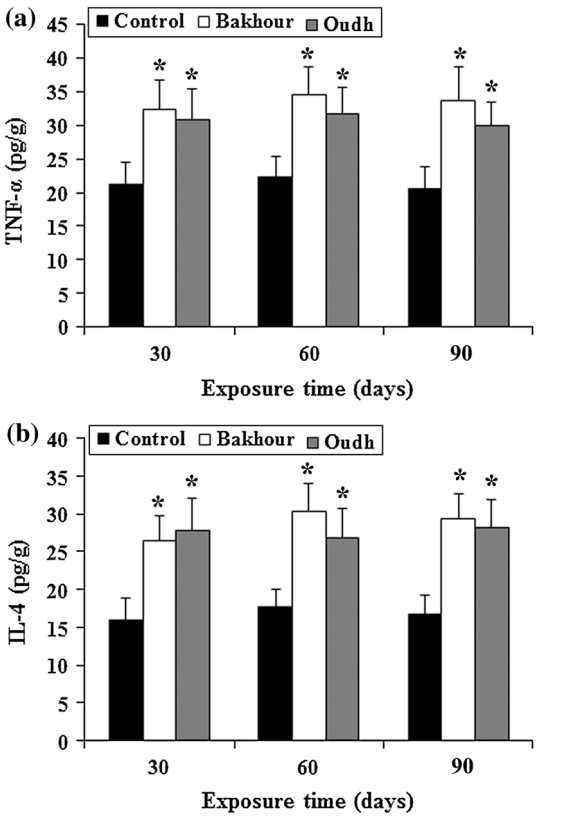

Fig. 5 TNF-α and IL-4 levels in the liver tissue of control and incense smoke exposed rats. Rats were exposed to smoke released by the burning of 4 g each of bakhour or oudh for 30, 60, or 90 days. The TNF-α and IL-4 levels in the liver tissue homogenates of exposed or unexposed control rats were measured by ELISA-based method. The data represent the mean ± SD ($N = 8$), *$p < 0.001$

induce CYP1A1, CYP1A2, and CYP1B1 genes in both lung and liver tissues of rats. Induction of these genes in these tissues indicates the effect of incense smoke in target and non-target tissues, and thus underscores its potential to cause widespread damage. Importantly, although the constitutive expressions of CYP1A1 and CYP1B1 were higher in the lung, the extent of induction was found to be greater in the liver tissues than in lung. Contrastingly, the constitutive expression of CYP1A2 was higher in liver, while the magnitude of induction was more in the lung. Thus, the extent of induction of these genes is contrary to their constitutive expressions in the liver and lung tissues. We observed a higher induction of CYP1A1 compared to CYP1A2 and CYP1B1 in both lung and liver tissues. This may suggest a more important role for CYP1A1 than CYP1A2 and CYP1B1 in the metabolic activation of PAHs, which is consistent with other studies [44, 45]. The sustained increase in mRNA levels of all the three studied enzymes over a period of 3 months indicates that the effect of incense smoke on these enzymes persists with the continuation in incense smoke exposure and underlines the chronic adverse health effects associated with the regular incense use. The PAHs are high affinity ligands of Aryl hydrocarbon receptors (AhR), which induce the genes containing the xenobiotic response elements. Genes induced by the PAH-AhR pathway includes CYP1A1, CYP1A2, GST, UGT-1, and CYP1B1 [30, 31]. It is likely that the incense smoke, due to its PAH content, followed similar pathway in the induction of CYPs in the lung and liver tissues. This study could only ascertain the effect of incense smoke on expression of CYPs; therefore. it is unclear whether the observed effect is due to a single component or the combinations of the components present in the incense smoke. In the present study, we did not notice any abnormal or behavioral changes in the rats to correlate with the changes at molecular level at least until the exposure duration was completed.

Previous studies have reported increased oxidative stress and inflammatory activities after exposure to incense smoke. Alveolar basal epithelial cells A549 exposed to incense smoke particulate matter exhibited increased oxidative stress, DNA damage, cell cycle arrest, cytoskeletal remodeling,

increased intracellular calcium, and induction of apoptosis [36, 46]. Importantly, these events are significantly mitigated when the cells are co-exposed to antioxidant, N-acetyl-l-cysteine. Increased secretion of IL-6, endothelin-1, and decreased NO production are reported in the human coronary artery endothelial cells in response to incense particulate matter exposure [20]. Arabian incense exposure of male Wister rats induced the ultra-structural changes in alveolar pneumocytes and the infiltration of neutrophils in pulmonary alveoli accompanied with degenerative and necrotic changes of the alveolar cells [47]. Exposure of A549 cells to Arabian incense upregulated the IL-8 and COX-2 inflammatory gene expression [21]. Consistent with the above data we observed increased oxidative stress, antioxidant, and inflammatory activities in the liver and lung tissues after incense smoke exposure as evident from the increased MDA, GSH, catalase activity, IL-4, and TNF-α. While the GSH levels are expected to be low under stress, the elevated GSH levels found in our study may possibly reflect an adoptive response by the cells to negate the increased oxidative stress, particularly in circumstances of chronic stimulus as is the case in our study where the rats were chronically exposed to incense smoke. A similar increase in the GSH levels as well as other antioxidant molecules and enzymes was reported when subjects are chronically exposed to cigarette smoke [48, 49]. Oxidative stress driven inflammation is a major cause of lung injury and the development of asthma, and other respiratory complications [50, 51]. Accordingly, incense smoke exposure has been found to be responsible for the non-malignant respiratory diseases including asthma and allergy [13, 52, 53]. Thus, increased oxidative stress and inflammation found in the lung tissues in our study and by others as a result of incense smoke exposure points to the widespread damaging effect of incense smoke [21, 36, 47].

In conclusion, we showed here for the first time the induction of CYP1A1, CYP1A2, CYP1B1 genes and their impact on oxidative stress and inflammatory activity in response to long-term incense smoke exposure. Furthermore, induction of CYPs together with an increase in oxidative stress and inflammation may promote the cancer pathogenesis as well as other clinical complications.

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Conflict of interest None

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