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RESEARCH ARTICLE

Deleterious effects of incense smoke exposure on kidney function and architecture in male albino rats

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Abstract

Context: Previous studies, including ours, have shown adverse effects of incense smoke on human health. However, the effect of incense smoke on kidney function and structure remains unknown.
Objective: To evaluate possible adverse effects of incense smoke on kidney function and architecture in albino rats after chronic exposure to Arabian incense.
Materials and methods: Emission characteristics including particle size distribution, volatile organic compounds (VOCs) and polycyclic aromatic hydrocarbons (PAHs) were determined by gravimetric and GCMS analyses. Kidney functional markers, oxidative stress and inflammatory markers were measured by standard or ELISA based procedures. Ultrastructural changes in kidney were examined by transmission electron microscope (TEM) and the gene expression of xenobiotic metabolizing enzymes including cytochrome P-450-1A1 (CYP1A1) and CYP1A2 were studied by real time PCR.
Results: Rats exposed to incense smoke demonstrated a significant increase in serum creatinine, uric acid, blood urea nitrogen (BUN), tissue malondialdehyde (MDA), tumor necrosis factor-alpha (TNF-α) and interleukin-4 (IL-4) levels and a significant decline in tissue reduced glutathione (GSH) and catalase activity. Incense smoke exposed rats also displayed marked ultrastructural changes in kidney tissue. Further, a significant increase in tissue gene expression of both CYP1A1 and CYP1A2 was noted in exposed rats.
Discussion: Changes to kidney functional markers and architecture appear to be mediated through augmented oxidative stress and inflammation.
Conclusion: Long-term exposure to incense smoke may have deleterious effects on kidney function and architecture. Though, inhalation is the rout of exposure, findings of this study underscore that incense smoke may also have an effect on non-pulmonary tissues.

Keywords

Incense smoke, inflammation, kidney, kidney architecture, oxidative stress, xenobiotic metabolizing enzymes

History

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Introduction

In the majority of South East Asian and Middle East nations incense burning is routinely practiced during religious rituals and to fragrance homes (Wang, 2007; Yeatts et al., 2012). Incense smoke typically contains particulate matter, CO, CO_2, NO_2 and SO_2, aldehydes, metallic elements, polycyclic aromatic hydrocarbons (PAHs) including benzo (a) pyrene (BaP), naphthalene, and fluoranthene and volatile organic compounds (VOCs) such as benzene, toluene, and xylene (Chiang et al., 2009; Chuang et al., 2011; Hwang et al., 2014; Wang, 2007). Arabian incense widely used in Middle Eastern nations is referred to as bakhour and oudh. Both of these incenses are extensively burnt to fragrance homes on daily basis. A wide variety of substances are used to produce bakhour, including sandal wood tree resin, agar wood, essential oils and perfumes, while oudh is a homogenously made agar wood from Aquilaria agallocha, which develops an aromatic smell due to fungal infection (Alokail et al., 2011; Wahab & Mostafa, 2007; Yeatts et al., 2012).

Recent studies have indicated that long term exposure to incense smoke may have adverse health effects. For example, incense smoke exposure has been linked to increased risk of developing cancer, allergic contact dermatitis, asthma and other respiratory complications (Al-Rawas et al., 2009; Friborg et al., 2009; Pan et al., 2014; Xie et al., 2014; Yang et al., 2009). Particulate matter generated in household

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burning of the incense is shown to be responsible for changes to heart rate variability and impaired endothelial function (Huang et al., 2014; Weber et al., 2011). Importantly, regular exposure to incense smoke is correlated with increased cardiovascular mortality (Pan et al., 2014). Likewise, exposure of human coronary artery endothelial cells to incense smoke increased the production of inflammatory mediators and endothelin-1 and decreased the NO formation (Lin et al., 2012). At the cellular level, incense smoke is described to induce genotoxicity, oxidative stress and inflammation (Chen & Lee, 1996; Chiang & Liao, 2006; Chuang et al., 2013; Cohen et al., 2013; Hussain et al., 2014; Lin et al., 2012; Navasumrit et al., 2008). We have previously shown that long term Arabian incense smoke exposure contributes to increased oxidative stress and inflammation in lung, liver and heart tissues of rats (Al-Attas et al., 2015; Hussain et al., 2014). We have also found that incense smoke exposure results in insulin resistance, dyslipidemia, altered spermatogenesis and sperm parameters in male Wister rats (Ahmed et al., 2013; Alokail et al., 2011). Considering the high susceptibility nature of kidney to oxidative stress, we suspected that chronic exposure to incense smoke may have deleterious effects on kidney functions and architecture. To test this possibility we exposed male albino rats to bakhour or oudh, the two commonly used well characterized types of incense used in Middle Eastern region (Ahmed et al., 2013; Al-Attas et al., 2015; Alokail et al., 2011; Al-Rawas et al., 2009; Dalibalta et al., 2015; Hussain et al., 2014; Yeatts et al., 2012), for extended durations and measured the kidney functional markers and tissue ultrastructural changes. Further, we examined the oxidative stress, inflammation and the genetic expression of xenobiotic metabolizing enzymes including CYP1A1 and CYP1A2 in kidney tissue to understand the possible underlying mechanism.

Methods

Animals

The Ethics Committee of the Experimental Animal Care Center, College of Science, King Saud University, Riyadh, Saudi Arabia approved the study. All experiments were performed in accordance with the set guidelines for the use and care of animals. Male Wistar albino rats aged 7–8 weeks, weighing 200–210 g, were obtained from the Animal Care Center, College of Pharmacy, King Saud University; Riyadh. 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*.

Emission characteristics of incense smoke

A set of three air samplers (AirMetrics, OR, US) with a flow rate of 5LPM were used to collect particulate matter ($<PM_{2.5}$ and $<PM_{10}$). Quartz filters were weighed before and after the incense burning using precision balance (Mettler-Toledo Inc., Zurich, Switzerland) to determine the particle mass of specific size. The VOCs were measured using Radiello® VOC samplers (Supelco Inc., PA). The PAHs associated with $PM_{2.5}$ were analyzed by Varian CP-3800 Gas Chromatograph with a model CP-8400 auto sampler interfaced to a 1200L Varian Quadrupole Mass Spectrometer by following the reported protocol of sample extraction (DRI, 2008; He et al., 2010; Zielinska et al., 2004) and standard procedure of chemical analysis (USEPA, 1997). All Radiello samples were analyzed by the thermal desorption cryogenic pre-concentration method followed by high-resolution gas chromatographic separation and mass spectrometric detection of individual compound. USEPA TO-15 (USEPA, 1999) and DRI SOP (DRI, 2010) methods were followed to quantify the concentrations of VOCs.

Exposure to incense smoke and sample procurement

After two weeks of acclimatization, rats were randomly divided into groups namely charcoal, 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 daily to whole body exposure of incense smoke by burning 4 g of respective incense on self-burning charcoal as described previously (Hussain et al., 2014). Rats from the charcoal group were exposed only to emissions from the burning charcoal. Eight rats from each group were anesthetized with pentobarbital sodium (35 mg/kg, IP) and euthanized by cervical decapitation after 30, 60 or 90 days from the commencement of the incense smoke exposure. To test the possible effects of emissions from the burning charcoal, an additional group of rats ($N = 8$) that were not exposed any combustion (control) were included in the study. Blood samples were collected from all the rats and the serum were separated for the estimation of creatinine, uric acid and BUN. Kidneys were excised and snap frozen in liquid nitrogen and stored at $-80°$ C until analyzed. For the analysis of biochemical parameters, tissues were homogenized in 100 mmol KH_2 PO_4 buffer containing 1 mmol EDTA (pH 7.4). Homogenates were centrifuged at 12 000$\times g$ for 30 min at 4 °C and the clear supernatants were separated and used for the measurement of MDA, catalase activity, GSH, TNF-α and IL-4 as described below. Total RNA was extracted from the tissues using RNAeasy mini kit (Qiagen, CA) and used for the gene expression analysis of CYP1A1 and CYP1A2 by real-time PCR.

Measurement of serum creatinine, BUN and uric acid

Serum creatinine was measured following the manufacturer's instructions (Crescent diagnostics, Jeddah, Saudi Arabia). Briefly, 15 µl of serum was added in duplicate to microplate wells followed by the addition of 100 µl each of reaction buffer containing sodium hydroxide and sodium borate and color reagent containing 1.2% picric acid. Change in absorbance at 495 nm was measured after 1 and 7 min. Creatinine levels were calculated from the standard curve generated by similarly processing the creatinine standards. The BUN was measured using the commercially available kit (Crescent Diagnostics, Jeddah, Saudi Arabia). Briefly, 5 µl of serum in duplicate was added to microplate wells followed by the addition of 150 µl each of urease enzyme and alkaline hypochlorite solution. Plate was incubated for 10 min at room temperature and the absorbance was measured at 620 nm. The BUN content in the sample was calculated from the

calibration curve constructed by similarly processing the urea standards. Uric acid was assayed in accordance with the manufacturer's instructions (Crescent diagnostics, Jeddah, Saudi Arabia) where 5 µl of sample in duplicates was mixed with 200 µl of working reagent containing reaction buffer, uricase enzyme and 2,4,6-tripyridyl-s-triazine coloring dye in microplate. Contents were incubated at room temperature for 30 min and the absorbance was measured at 590 nm. Uric acid levels were calculated from the calibration curve constructed by similarly processing the uric acid standards.

Measurement of MDA

The MDA content was determined according to Draper & Hadley (1990). Briefly, 0.5 ml of kidney tissue extract supernatant was mixed with 1 ml of trichloroacetic acid solution and centrifuged at 2500 *g* for 10 min. The clear supernatant [0.5 ml] of tissue homogenate 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 measured at 532 nm, and the concentration of MDA was calculated and expressed as nmol/mg protein.

Measurement of catalase activity

The catalase activity in kidney tissue was assayed according to the method by Aebi (1984). A volume of 50 µl of the clear supernatant of tissue homogenate was added to 3 ml reaction mixture containing 0.05 M Tris buffer, 5 mM EDTA (pH 7.0) and 10 mM H_2O_2 in 0.1 M potassium phosureaphate buffer (pH 7.4). The rate of change in absorbance per minute at 240 nm was recorded. Catalase activity was expressed in terms of µmoles H_2O_2 consumed/min per milligram of protein.

Measurement of GSH

The measurement of GSH in kidney tissue was carried out according to the procedure reported by Owen (1980). Briefly, 100 µl of clear supernatant of 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 kidney tissue were determined by competitive ELISAs following the manufacturer's instructions (My Biosource, CA). Briefly, the tissue supernatant and the TNF-α-HRP conjugate or IL-4-HRP conjugate were added to the plate pre-coated with the TNF-α or IL-4 antibodies, respectively. Plates were incubated for 1 h, washed and incubated with the HRP substrate, 3,3-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.

Tissue preparation and ultra-structural study

Immediately after resection, kidney was sliced into small sizes (1 mm^3) and the slices were fixed in 3% buffered glutaraldehyde solution for 4 h at 4 °C. Tissue specimens were then post-fixed in 1% osmium tetroxide (OsO_4) for 90 min. Dehydration of the fixed tissue was performed using ascending grades of ethanol and transferred to resin via propylene oxide. After impregnation with the pure resin, tissues specimens were embedded in the same resin mixture. Ultra-thin sections of silver shades (60–70 nm) cut on an ultra-microtome (Leica, UCT) with a diamond knife were placed on copper grids and stained with uranyl acetate (20 min) and lead citrate (5 min). Stained sections were observed under TEM (JEOL JEM-1011) operating at 80 kV.

Real-time PCR

The relative expressions of CYP1A1 and CYP1A2 genes in kidney tissue of control and incense smoke exposed rats were evaluated by real-time PCR. One microgram of total RNA extracted from the kidney tissue was reverse transcribed to cDNA using QuantiTect Reverse Transcription Kit (Qiagen, CA). Real-time PCR was performed on CFX96 Real-Time PCR system (Bio-Rad Laboratories, Hercules, CA) in a 96 well plate format. The reaction mix consisted of cDNA equivalent to 100 ng of total RNA, SYBR green master mix (Kappa Bioscience, MA), and 10 µmol each of forward primer, 5′-CCA TGA CCA GGA ACT ATG GG-3′ and reverse primer, 5′-TCT GGT GAG CAT CCA GGA CA of CYP1A1 and forward primer, 5′-CGCATTGGCTCCACA CCCGTG-3′ and reverse primer, 5′-ACCGATTCCACCAC CTGGTTGACT-3′ of CYP1A2 genes. The PCR was carried out with an initial cycle consisting 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.

Statistical analysis

Data were analyzed using the IBN SPSS version 21.0 (IBM, Armonk, NY). Significance was set at $p < 0.05$. Biochemical parameters were expressed as mean ± standard deviation (SD). The analysis of variance (ANOVA) was used to compare the various markers in different treatment groups followed by Tukey's honestly significant difference (HSD) *post hoc* test.

Results

Emission characteristics of incense smoke

The size and mass of particles, and PAHs present in two types of incense smoke are presented in Table 1. The PM2.5 were the highest particle fraction and mass present in both the incense types studied, while the particles with size range between 2.5 and 10 µm were negligible (Table 1). With regard to PAHs associated with $PM_{2.5}$ in bakhour and oudh, 9-fluorenone (41.8 and 38.7 mg/kg, respectively) demonstrated the highest emission factor followed by benzo[a]pyrene (10.5 and 9.4 mg/kg, respectively), phenanthrene (9.6 and

Table 1. Particle mass (mg/g) and PAHs (mg/kg) present in bakhour and oudh smoke.

	Bakhour	Oudh
$PM_{2.5}$	120	105
PM_{10}	3	2.5
9-Fluorenone	41.8 ± 6.39	38.6 ± 0.16
Benzo[a]pyrene	10.5 ± 0.08	9.8 ± 0.16
Phenanthrene	7.0 ± 1.08	9.5 ± 1.66
Anthracene	1.5 ± 0.23	2.8 ± 0.49
Fluoranthene	2.5 ± 0.39	5.7 ± 1.00
Pyrene	4.8 ± 0.74	8.3 ± 1.45
Benz[a]anthracene	1.5 ± 0.23	2.8 ± 0.52
Benzo[b]fluoranthene	0.9 ± 0.14	1.2 ± 0.21
Indeno[123-cd]pyrene	0.2 ± 0.03	ND
Benzo(ghi]perylene	0.8 ± 0.14	0.7 ± 0.09

Table 2. The VOCs present in the bakhour and oudh smoke.

VOCs in g/kg	Bakhour	Oudh
n-Hexane	0.018 ± 0.001	0.014 ± 0.001
Benzene	1.8 ± 0.16	1.6 ± 0.13
Cyclohexane	0.18 ± 0.02	0.12 ± 0.01
Toluene	1.14 ± 0.07	1.12 ± 0.09
Ethyl benzene	0.16 ± 0.01	0.20 ± 0.02
m,p-Xylene	0.40 ± 0.05	0.36 ± 0.04
Styrene	1.4 ± 0.13	1.5 ± 0.13
1,2,4-Trimethyl benzene	0.48 ± 0.03	0.43 ± 0.06

10.4 mg/kg, respectively), pyrene (8.3 and 7.6 mg/kg, respectively), fluoranthene (5.7 and 4.8 mg/kg, respectively) and benz[a]anthracene (1.5 and 1.8 mg/kg, respectively) (Table 2). The different VOCs present in bakhour and oudh smoke are listed in Table 2. Among VOCs, it can be seen that benzene has the highest emission factor among VOHs for both bakhour and oudh smoke (1.8 and 1.6 g/kg, respectively). Apart from benzene, toluene (1.2 and 1.1 g/kg, respectively) and styrene (1.4 and 1.5 g/kg, respectively) have shown higher emission factors compared to other VOCs.

Kidney functional markers

The levels of serum creatinine, BUN and uric acid in charcoal, bakhour and oudh incense smoke exposed rats are presented in Table 3. A significant increase in creatinine levels was observed in both bakhour and oudh exposed rats as compared to those in charcoal exposed rats. Bakhour and oudh exposed rats displayed a greater effects on creatinine levels after 30 days of exposure than 60 and 90 days exposures. However, bakhour and oudh had identical effects on creatinine levels after 60 and 90 day time-point exposures. Rats exposed to both the incense materials had a significantly elevated BUN after 30, 60 and 90 days of exposures as against charcoal exposed rats. No significant difference in the effect of bakhour on BUN was noted among three time points of exposures. Further the effect of bakhour on BUN was comparable to that of oudh at the three measured time points. Consistently, rats exposed to bakhour or oudh had a significantly elevated uric acid content after 30, 60 and 90 days of exposures as against charcoal exposed rats. No significant difference in the effect of bakhour on uric acid was noted among three time points of exposures. Further the effect of bakhour on uric acid was comparable to that of oudh at the three measured time points.

Oxidative stress markers

The kidney MDA, GSH and catalase activity levels in charcoal, bakhour and oudh exposed rats are presented in Table 4. Bakhour or oudh exposed rats had significantly elevated MDA, and significantly decreased GSH and catalase activity levels after 30 days of exposure. Further, compared to charcoal, the levels of MDA remained significantly elevated and GSH and catalase levels remained significantly declined with the continuous exposure for up to 60 and 90 days. Between the two types of incense exposures, the bakhour smoke exerted a significantly greater effect than oudh on MDA levels when compared after 30, 60 or 90 days of exposure. Likewise, the effect of bakhour on GSH was significantly different than oudh. The effect of bakhour on catalase activity was comparable to that of oudh after 30 days of exposure while it was greater after 60 and 90 days of exposures.

Inflammatory markers

The TNF-α and IL-4 levels in charcoal and incense smoke exposed rats are provided in Table 5. Bakhour smoke exposure of rats for 30, 60 or 90 days resulted in a significant increase in TNF-α and IL-4 levels as matched to those in charcoal emission exposed control rats. Bakhour exposure led to a significantly higher TNF-α levels after 30 days of exposure than 60 and 90 of exposure in relation to oudh exposure. There were significantly elevated IL-4 levels in rats exposed to bakhour than in rats exposed to oudh at all the three time durations tested. Among the studied inflammatory markers, bakhour exposure had a greater effect on IL-4 than on TNF-α.

Effect of charcoal emission

To test the possible effects of charcoal emissions, we measured the kidney functional, oxidative stress and inflammatory markers in rats exposed to fresh air (control). The serum creatinine, BUN and uric acid levels and kidney MDA, GSH, catalase, TNF-α and IL-4 levels in charcoal emission exposed and control rats are presented in Table 6. We found that among the studied markers only catalase and TNF-α levels in rats exposed to charcoal emissions were significantly higher than those in control rats.

Ultra-structural changes

Ultra-structural changes in kidney tissues of unexposed control, charcoal, bakhour and oudh exposed rats are presented in Figure 1. Compared to unexposed control (Figure 1A), no significant changes in the tissue architecture was found in rats exposed only to charcoal emission (Figure 1B). Contrastingly, significant nephropathy changes were observed in the corticomedullary region of bakhour and oudh exposed rats (Figure 1C and 1D, respectively). Nephropathies were characterized by glomerular damage, including dilatation of

Table 3. Serum functional markers in charcoal, bakhour or oudh exposed rats.

	30 days			60 days			90 days		
Markers	Charcoal ($N=8$)	Bakhour ($N=8$)	Oudh ($N=8$)	Charcoal ($N=8$)	Bakhour ($N=8$)	Oudh ($N=8$)	Charcoal ($N=8$)	Bakhour ($N=8$)	Oudh ($N=8$)
Creatinine (mg/dl)	0.8 ± 0.1	1.2 ± 0.2**	1.3 ± 0.2**	0.7 ± 0.1	1.0 ± 0.2*	1.3 ± 0.3*	0.5 ± 0.1	1.0 ± 0.3*	1.1 ± 0.3*
BUN (mg/dl)	37.0 ± 2.6	45.3 ± 4.2*	46.6 ± 4.2*	36.4 ± 2.2	47.4 ± 6.4*	52.5 ± 8.3*	36.0 ± 2.4	44.4 ± 9.1*	49.1 ± 7.9*
Uric acid (mg/dl)	1.3 ± 0.1	1.7 ± 0.2*	1.8 ± 0.2*	1.4 ± 0.1	1.7 ± 0.2*	1.8 ± 0.3*	1.4 ± 0.1	1.7 ± 0.3*	1.7 ± 0.2*

BUN: blood urea nitrogen.
*$p<0.001$.
**$p<0.0001$.

Table 4. Kidney oxidative stress markers in charcoal, bakhour or oudh exposed rats.

	30 days			60 days			90 days		
Markers	Charcoal ($N=8$)	Bakhour ($N=8$)	Oudh ($N=8$)	Charcoal ($N=8$)	Bakhour ($N=8$)	Oudh ($N=8$)	Charcoal ($N=8$)	Bakhour ($N=8$)	Oudh ($N=8$)
MDA (nmol/mg)	3.9 ± 0.9	6.2 ± 1.0**,†	5.5 ± 0.6**	4.1 ± 0.9	6.3 ± 1.1**,§	5.5 ± 0.8**	4.1 ± 1.0	6.3 ± 1**,†	5.6 ± 0.7*
GSH (nmol/mg)	19.2 ± 1.4	16.6 ± 1.2**	16.3 ± 1.2*	19.0 ± 1.3	16.1 ± 1.7**	17.2 ± 0.7*	19.06 ± 1.3	15.8 ± 1.2**	17.4 ± 0.9*
Catalase (μmol/min/mg)	27.1 ± 2.28	21.5 ± 3.2**	23.3 ± 2.5**	27.9 ± 2.3	23.4 ± 2.2**	23.9 ± 3.6*	25.5 ± 3	21.6 ± 2**	22.7 ± 1.7*

MDH: malondialdehyde, GSH: reduced glutathione.
*$p<0.05$.
**$p<0.01$.
†Significantly different from oudh group ($p<0.05$).
§Significantly different from oudh group ($p<0.01$).

Table 5. Kidney inflammatory markers in charcoal, bakhour or oudh exposed rats.

	30 days			60 days			90 days		
Markers	Charcoal ($N=8$)	Bakhour ($N=8$)	Oudh ($N=8$)	Charcoal ($N=8$)	Bakhour ($N=8$)	Oudh ($N=8$)	Charcoal ($N=8$)	Bakhour ($N=8$)	Oudh ($N=8$)
TNF-α (pg/g)	16.8 ± 2.5	21.0 ± 2.9**	21.6 ± 3.5*	16.0 ± 1.7	21.1 ± 2.5**	21.3 ± 2.9**	16.5 ± 2.1	21.5 ± 2.5**	20.6 ± 2.2**
IL-4 (pg/g)	40.7 ± 3.2	55.1 ± 8.7†	53.2 ± 8.6**	41.1 ± 3.4	51.1 ± 7.5**	48.4 ± 5.5*	41.5 ± 2.4	57.0 ± 7.0†	52.1 ± 7.0**

TNF-α: tumor necrosis factor-alpha, IL-4: interleukin-4.
*$p<0.05$.
**$p<0.01$.
†$p<0.001$.

Table 6. Functional, oxidative stress and inflammatory markers in control and charcoal exposed rats.

Parameters	Control ($N=8$)	Charcoal ($N=8$)
Creatinine (mg/dl)	0.75 ± 0.1	0.5 ± 0.1
BUN (mg/dl)	38.1 ± 1.9	36.0 ± 2.4
Uric acid (mg/dl)	1.2 ± 0.1	1.4 ± 0.1
MDA (nmol/mg)	3.5 ± 1.0	4.1 ± 1.0
GSH (nmol/mg)	18.6 ± 3	19.06 ± 1.3
Catalase (μmol/min/mg)	32 ± 6	25.5 ± 3*
TNF-α (pg/g)	12.4 ± 1.8	16.5 ± 2.1*
IL-4 (pg/g)	39.3 ± 3.6	41.5 ± 2.4

BUN: blood urea nitrogen, MDA: malondialdehyde, GSH: reduced glutathione, TNF-α: tumor necrosis alpha, IL-4: interleukin-4
*$p<0.05$.

glomerular blood vessels, exfoliation and shedding of proximal tubular cells into the tubular lumen, proteinaceous cast formation, thickened basement membrane and interstitial inflammation as evidenced by abundant interstitial monocytes. Furthermore, we also noticed degenerated mitochondria, vacuoles, mesangial expansion and cell debris.

Induction of CYP1A1 and CYP1A2 genes in kidney

Fold change in kidney CYP1A1 and CYP1A2 mRNA levels in charcoal and incense smoke exposed rats are shown in Figure 2. Exposure to bakhour led to 12, 11 and 11.7-fold induction of CYP1A1 mRNA after 30, 60 or 90 days, respectively, while oudh exposure for identical times contributed to 11, 10 and 11-fold increase, respectively, compared to charcoal emission exposed rats . The CYP1A2 mRNA exhibited a 10, 11.5 and 9-fold increase after 30, 60 and 90 days of bakhour exposure, respectively. Compared to control, exposure to oudh resulted in 9, 10 and 10.5-fold upregulation of CYP1A2 mRNA at 30, 60 and 90 days of exposure. Additionally, we compared the effects of charcoal emissions on CYP1A1 and CYP1A2 with that of control. The fold change in the mRNA levels in control and charcoal emission exposed rats are provided in Figure 3. Charcoal emission significantly increased the expression of both CYP1A1 and CYP1A2 mRNA levels compared to that in control rats.

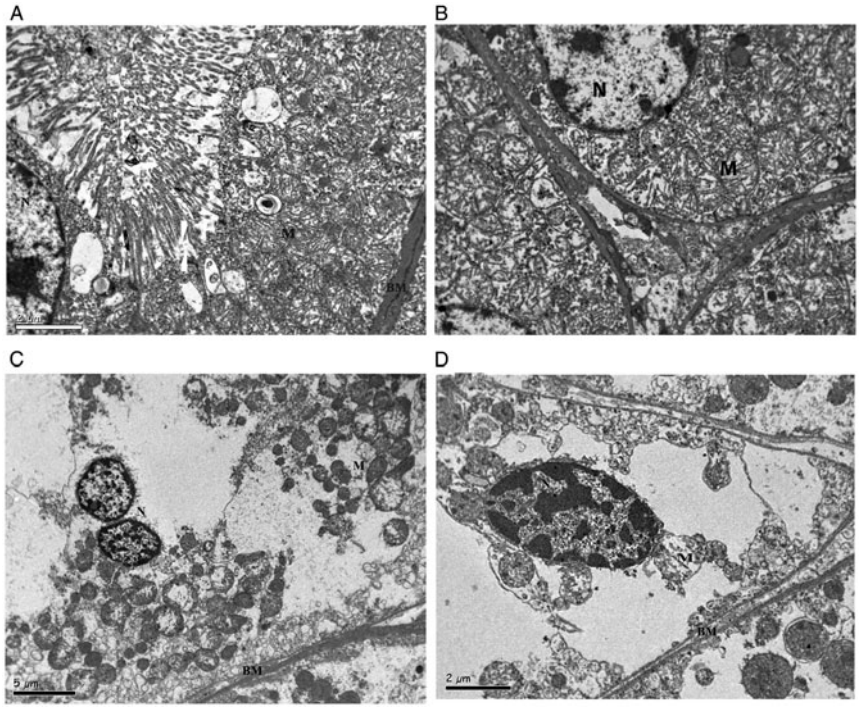

Figure 1. Ultra-structural changes in rat kidney: Rats were exposed to bakhour or oudh incense for 90 days. Kidneys were dissected out and ultra-structural changes in unexposed control (A), charcoal emission (B), bakhour (C) or oudh (D) exposed rats were evaluated by transmission electron microscope as described in methodology. Mitochondria (M), basement membrane (BM), filtration units (F) and nucleus (N) could be seen.

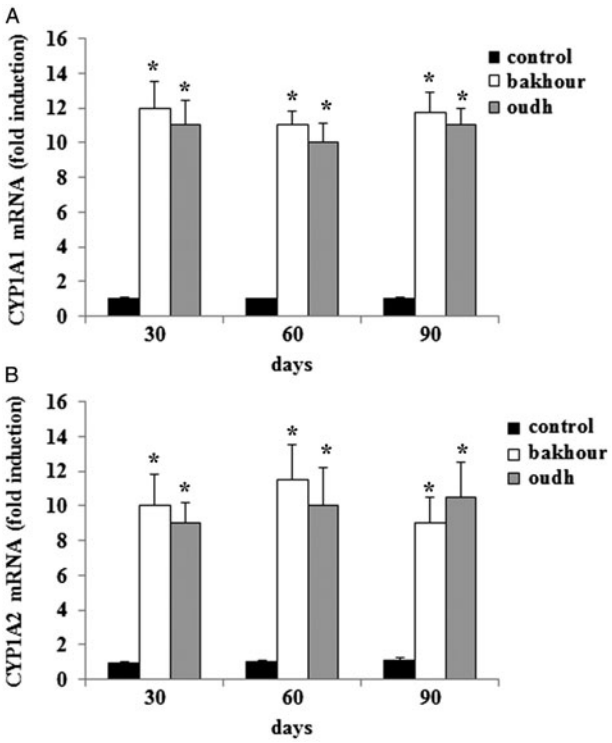

Figure 2. Inductions of CYP1A1 and CYP1A2 mRNAs in rat kidney: Rats were exposed to bakhour or oudh incense for 30, 60 or 90 days. Total RNA was extracted from the kidney tissues of exposed and charcoal emission exposed rats at the indicated times. Relative expressions of CYP1A1 and CYP1A2 mRNAs were measured by real time quantitative PCR. The data presented are the mean ± SD ($N = 8$), $^*p < 0.001$.

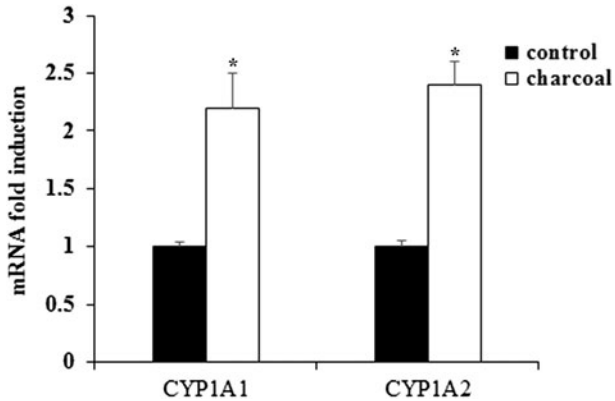

Figure 3. Induction of CYP1A1 and CYP1A2 mRNAs in rat kidney: Rats were left unexposed (control) or exposed to burning charcoal for 90 days. Total RNA was extracted from the kidney tissues and the relative expressions of CYP1A1 and CYP1A2 mRNAs were measured by real time quantitative PCR. The data presented are the mean ± SD ($N = 8$), $^*p < 0.05$.

Discussion

Increasing number of reports has hinted at the adverse effects of short term as well as long term exposure to incense smoke or to its particulate or gaseous constituents. These studies have shown the incense smoke exposure to negatively affect the normal physiological functions of lung and non-pulmonary organs such as liver and heart and thereby to increase the risk of developing chronic diseases such as cancer, cardiovascular and respiratory diseases. In this study we evaluated the possible unfavorable effects of incense smoke on kidney function and tissue architecture. We further examined the possible underlying mechanisms in the negative modulation of kidney function by incense smoke. In incense smoke exposed rats we found a significant increase in all the tested functional markers of kidney including creatinine, BUN and uric acid which clearly demonstrates the disturbed normal physiological functions of kidney and suggests the negative modulating effects of incense smoke on kidney functions. Importantly, increased levels of these functional markers were found within 30 days of incense smoke exposure and

remained significantly elevated at least up to 90 days. This underscores the sustained effects of incense smoke on kidney functions with the continuous exposure. Further, we found no significant differences between the effects of bakhour and oudh on the studied functional parameters of kidney. While this is the first study to identify the detrimental effect of incense smoke on kidney function, our findings however are consistent with the studies where the negative effects of other established environmental factors on kidney functions are reported. For example the incidence of chronic kidney diseases is well described in cigarette smokers (Noborisaka et al., 2013; Jain & Jaimes, 2013). Also, widespread damage to kidney tissue with distinct nodular glomerulosclerosis is observed in cigarette smokers (Batal et al., 2014). Besides, impaired kidney development and function in response to fetal smoke exposure are reported in humans and animal studies (Al-Odat et al., 2014; Kooijman et al., 2015). Mice treated with environmental contaminant such as carbon tetrachloride exhibited significant kidney dysfunction, increased serum levels of BUN, and uric acid (Ma et al., 2014). Co-exposure of arsenic, cadmium and cigarette smoke is shown to cause kidney toxicity in humans (Arain et al., 2015). Likewise, kidney injury is reported in individuals who smoked synthetic cannabinoids (Buser et al., 2014). These studies corroborate with our finding of unfavorable effects of incense smoke on kidney function. In addition to measuring the serum functional markers, we examined the changes to kidney architecture in response to incense smoke exposure. Distinct nephropathy changes in corticomedullary region including glomerular damage with dilated blood vessels, exfoliation and shedding of proximal tubular cells into the tubular lumen were observed in incense smoke exposed animals. Further, degenerated mitochondria, vacuoles, mesangial expansion and cell debris were noted in the exposed rats. Collectively, these structural changes to the kidney clearly demonstrate the tissue degenerative effects of incense smoke.

To understand the possible mechanistic events triggering the kidney dysfunction in incense smoke exposed rats, we examined the oxidative stress and inflammation in kidney tissue. Usually, the reactive oxygen species (ROS) produced in the body are negated by the antioxidant defense system, thereby maintaining a healthy balance between oxidant and antioxidant states. Under disturbed physiological conditions however, increased generation of ROS and/or depletion of antioxidants results in an impaired clearance and accumulation of ROS, leading to oxidative stress which underlies the pathophysiology of numerous chronic diseases. Additionally, continued oxidative stress can trigger the infiltration of inflammatory mediators which in turn can lead to inflammation of affected tissue. Persistence of inflammation can further exacerbate the oxidative stress induced complications (Machowska et al., 2015; Martinon, 2010). Previous studies have implicated the oxidative stress and inflammation as major cellular changes promoting kidney damage. For instance, cysplatin induced kidney injury is shown to be mediated by increased oxidative stress and inflammation (Malik et al., 2015; Panxw et al., 2015). Besides microbial and pathological challenges, a number of environmental toxicants have been shown to alter the levels of oxidants and antioxidants in kidney (Cooke et al., 2007; Mima, 2013; Nerpin et al., 2012; Ozbek, 2012). Despite its increasing recognition as a potential environmental pollutant, incense smoke has not yet been tested for its ability to induce oxidative stress in kidney. In the present study, we found increased MDA and decreased catalase activity and GSH levels in the rats after 30 days of incense smoke exposure. Further, the effect of incense smoke sustained with the continued exposure at least up to 90 days as the levels of oxidative stress markers measured after 60 and 90 days were comparable to those found after 30 days of exposure. This suggests the increased generation of ROS and thereby the oxidative stress inducing capacity of incense smoke in kidney. These observations are supported by the findings of reduced levels of antioxidants catalase and GSH in the incense smoke exposed rats. This possibly reflects the depletion of these antioxidants in the process of continuous quenching of ROS due to their sustained and increased production in kidney of incense smoke exposed rats. Alternatively, incense smoke may have exerted the direct effect on catalase gene expression and also on gene expression of glutathione reductase, which catalyzes the conversion of oxidized glutathione to its reduced state. We also observed increased levels of inflammatory mediators in kidney tissues of smoke exposed animals demonstrating their increased infiltration and reflecting heightened immunological response to increased oxidative stress. This further substantiates the prooxidant nature of incense smoke. Several of the incense smoke constituents such as PAHs, heavy metals and particulate fractions are also present in the established environmental pollutants wherein they are shown to exert oxidative stress in kidney. For example, exposure of heavy metals such as lead and cadmium from the polluted water and air is shown to modify the oxidative stress markers in human kidney (Garçon et al., 2007; Huang et al., 2013). Rats exposed to industrial dust fiber amosite exhibited increased oxidative stress and renal fibrosis (Boor et al., 2009). Likewise, nanosized particulate fraction of diesel exhaust is reported to increase the oxidative stress in rat kidney (Nemmar et al., 2010). Rats exposed to cigarette smoke had elevated oxidative stress markers, reduced antioxidants and advanced tissue degeneration in kidney (Cigremis et al., 2004; Ramesh et al., 2010). Also exposure to nose only cigarette smoke increased the production of ROS and suppressed antioxidant defense system in kidney through decreased glutathione (GSH), GSH-Px, superoxide dismutase (SOD) and catalase activity (Raza et al., 2013). The BaP, present in cigarette smoke, grilled foods, automobile exhaust, and biomass smoke is shown to potently induce the oxidative stress and inflammation in kidney (Kim & Lee, 1997). Corroborating with the above experimental evidences, the BaP as well as other PAHs are the major constituents found in the smoke from bakhour and oudh used in this study. Thus, it is likely that one or more of these incense smoke constituents presumably responsible for the observed oxidative stress in the exposed rats.

To understand the possible mechanism operative in the induction of oxidative stress by incense smoke, we examined the expression of phase I xenobiotic metabolizing enzymes including CYP1A1 and CYP1A2. Incense smoke contains significant amounts of BaP, either airborne or particle bound (Chiang et al., 2009; Chuang et al., 2011; Wang, 2007).

The BaP is metabolized by phase I enzymes such as CYP1A1, CYP1A2, CYP1B1 and CYP2E1 resulting in the generation of reactive and unstable intermediary metabolites which are subsequently detoxified by phase II enzymes (Harrigan et al., 2006; Ioannides & Lewis, 2004; Kim et al., 1998). Importantly, BaP enhances its own metabolism by inducing the expression of these CYP enzymes (Harrigan et al., 2006; Pushparajah et al., 2008). Thus, the relative expression of phase I and phase II metabolizing enzymes determine the ratio of bioactivated to detoxified metabolites. Accordingly, impaired detoxification due to the altered expression of phase I and/or phase II enzymes may lead to accumulation of these reactive and unstable metabolites resulting in oxidative stress (Shimada, 2006). In this study, we found significant upregulation of CYP1A1 and CYP1A2 gene expressions in kidney of the incense smoke exposed rats. This also correlates well with the presence of BaP in the studied incense types. Consistent with our findings, previous studies have described a positive correlation between induction of CYPs and oxidative stress in response to environmental toxicants (Al-Arifi et al., 2012; An et al., 2011; Gentner & Weber, 2012; Raza et al., 2013; Tsuji et al., 2011). Importantly these toxicants like incense smoke, have considerable amounts of BaP as an active agent. Thus, it can be postulated that induction of CYPs by BaP or other constituents either alone or in combination therein constitutes the mechanistic event in the observed increase in oxidative stress, inflammation, kidney dysfunction and tissue degeneration in incense smoke exposed rats. 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 (Alexander et al., 1997; Sutter & Greenlee, 1992). It is likely that the incense smoke, due to its PAH content, followed similar signaling pathway in the induction of CYPs in kidney.

In this study we also tested whether the charcoal emissions per se had any effects on the studied parameters as well as on the kidney ultrastructure. We found that charcoal emissions showed significant effects on catalase and TNF-α levels and also on the CYP1A1 and CYP1A2 mRNA levels compared to those in the rats exposed to fresh air. No significant changes in other parameters and in the tissue architecture were noted in the charcoal emission exposed rats. Despite the significant modulating effects of charcoal on some of the studied markers, the effects of bakhour or oudh were found to be much higher in magnitude.

Conclusion

We showed here the deleterious effects of incense smoke on kidney function and architecture. These effects appear to be driven by the capacity of incense smoke to induce oxidative stress and inflammation. We further proposed the increased expression of CYP enzymes by incense smoke as a possible underlying mechanism involved in the induction of oxidative stress.

This is the first study to demonstrate the effect of incense smoke on kidney function and structure. Although inhalation is the rout of exposure, observations of this study indicate that non-pulmonary organs also are susceptible to environmental toxicants such as incense smoke. The finding of adverse effects of incense smoke in animal system made here have considerable relevance to humans considering the widespread use of incense, particularly in Middle Eastern and South East Asian countries Taken together, this study significantly advances our understanding of the mechanisms underlying the incense smoke induced toxicity in tissues other than lung.

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Declaration of interest

Authors report no declarations of interest. This study was supported by the Research Chair for Biomedical Applications of Nanomaterials, Deanship of Scientific Research, King Saud University, Riyadh, Saudi Arabia.

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