World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

The toxic effect of chemicals from burning incense (Bakhour) on immune system

Rawan Alzahrani *, Nawal Helmi and Israa Hakeem

Department of Biochemistry, College of Science, University of Jeddah, Jeddah, Saudi Arabia.

World Journal of Advanced Research and Reviews, 2025, 25(02), 1681-1692

Publication history: Received on 06 January 2025; revised on 13 February 2025; accepted on 16 February 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.25.2.0523

Abstract

Outdoor air pollution is a significant environmental health concern, linked to chronic respiratory diseases, heart conditions, and immunosuppression. One common indoor air pollutant is Bakhour, a fragrant incense burned in many homes. Despite its widespread use, little research has explored the potential health risks associated with Bakhour smoke exposure.

This study investigated the toxic effects of Bakhour smoke on the immune system. Three popular types of Bakhour (Oud, Mabthoth, and Mastic) were burned for four hours daily for two weeks, exposing 20 rats to the smoke. Weight, blood serum antibody levels (Immunoglobulin G and M), and spleen function were measured.

Results revealed that exposure to Bakhour smoke damaged spleen cells and led to immunosuppression, with the most severe effects observed in rats exposed to Mabthoth. Additionally, the study found a correlation between Bakhour smoke exposure and antibody formation.

To reduce exposure to Bakhour emissions, the study recommends minimizing burning time, increasing ventilation, and avoiding the use of charcoal. These findings highlight the importance of understanding the health risks associated with Bakhour smoke and taking steps to mitigate its impact on public health.

Keywords: Immunity; Incense; Oud; Mabthoth; Mastic; Air pollution

1. Introduction

The Arabic term "Bakhour" refers to perfumed wood bricks that are typically burned on charcoal or incense burners to produce a potent fragrance and dense smoke. However, incense is burned to welcome guests and to promote social harmony and serenity during weddings, religious festivities, family gatherings, and formal occasions [1].

Arabian Bakhour is a mixture of many different natural ingredients, such as fragrant woodchips from agar wood (Oud), herbs, flowers, musk, and sandalwood that has been impregnated with scented oils. With the help of tiny charcoal bricks, the incense burns slowly and produces a rich and fragrant smoke [2].

In addition to sulfur dioxide (SO2), formaldehyde (HCHO) and hydrogen sulfide, this smoke was found to contain particulate matter (PM), polycyclic aromatic hydrocarbons (PAHs), carbon monoxide (CO), nitrogen oxides (NOX), and hydrogen sulfide (H2S). Such contaminants are easily accumulated indoors particularly when there is insufficient ventilation [2].

* Corresponding author: Rawan Alzahrani

Additionally, it was discovered that most of these chemicals were created in quantities greater than that of tobacco smoke, in part because of emissions from charcoal briquettes. It had been established that burning coal to create the Bakhour smoke increased the concentration of sulfur dioxide in air [2].

This study's objective is to examine the subsequent health effects of these elements and their relationship to the immune system, by the identification of certain Immunoglobulin G and M (IgG and IgM) cells in the blood. Then conducted numerous tests and analyses on the rat spleen [3,4].

The monoterpenoids alpha-pinene, limonene, and myrcene as well as sesquiterpenoids are the main components. Boswellic acid, a triterpenoid credited with a variety of bioactivities, is the resin's main component. There are numerous diterpenoids in the resin as well. The resin mostly contains galactose, arabinose, xylose, and uronic acid [5].

When used repeatedly or over an extended period of time, incense consumption may present some health hazards. Because odorants can generate fresh during burning, using incense poses a unique challenge to scent research.

Scientific studies have particularly increased awareness over the past few decades that burning incense should be regarded a risky behavior for human health [6]. We hypothesize the chemicals compounds release from Bakhour smoke may effect on immune system.

Burning Bakhour is a custom in the Kingdom of Saudi Arabia and it is used almost daily to incense the air in house. There are also special types for occasions.

Due to its frequent use, the importance of searching for Bakhour smoke lies in several factors: Understanding of the nature of Bakhour smoke that individuals are frequently exposed to and potential impacts on the environment and their health. And raising awareness of the immune damages associated with inhaled Bakhour smoke.

2. Material and methods

2.1. Bakhour Samples

Three Bakhour samples were obtained and used in the study from domestic producer in Kingdom of Saudi Arabia. The types of Bakhour used in the study are Oud, Mabthoth and Mastic. The choice of the Bakhour types was based on their Price and popularity in the country.

2.2. Animals

Twenty Specific-pathogen-free albino Wistar rat (male, months of age (60 days) is considered an adult, weights ± 180g) were purchased from Animal House Unit, King Abdulaziz University. All rats had ad libitum access to standard rodent chow and filtered water.

2.3. Ethical Approval

All animal experimentation was approved by the Medical Scientific Foundation for Research and Development (MSF) of the King Abdulaziz University, Jeddah, Saudi Arabia. (Reference Number: p100-2023).

2.4. Chemicals and kits

Kits used for quantitative determination of different parameters were purchased from Mybiosource, California, (USA).

The materials that were used in this research: Diethyl ether (Honeywell Riedel-de Haën) Formalin, Paraffin and Leishman's stain (Sigma Aldrich), Phosphate Buffered Saline (UFC Bio), Ethanol (Fisher Chemical), Hematoxylins and Eosin Y (Thermo Scientific), Xylene (Central Drug House).

2.5. Experimental Protocol

This study included 20 rats that were divided into four groups based on weight, Control group and the remaining groups exposed to different types of Bakhour smoke. (G1) Group 1 (Control, fresh air), (G2) Group 2 (Oud), (G3) Group 3 (Mabthoth); (G4) Group 4 (Mastic).

Rats were housed separately to avoid cross exposure of incense smoke. After a week of acclimatization, rats were exposed to 320 grams of Bakhour for 2 weeks, at a rate of 32 grams/day in an exposure chamber for each group. Incense smoke was generated by placing a piece of incense on an electronic censer without using charcoal to focus on the damage of incense alone.

Each group was exposed to a type of incense for 4 separate hours a day, with 30 minutes of exposure to incense smoke and an hour of exposure to fresh air (8 periods), alternating until the specified time was completed. The incense burner was cleaned and a new amount of incense was placed at each interval.

Tow rats from the 2&3 group died after 7 and 10 days from the commencement of the incense smoke exposure. Blood samples were collected from dead rats and stored at -80°C until analyzed. Postmortem examination of the dead rat showed no medical causes. After the final day of exposure, blood was collected by retro-orbital bleeding, and spleens were removed for every 3 rats from each group. Finally all rats were sacrificed (Figure 1).

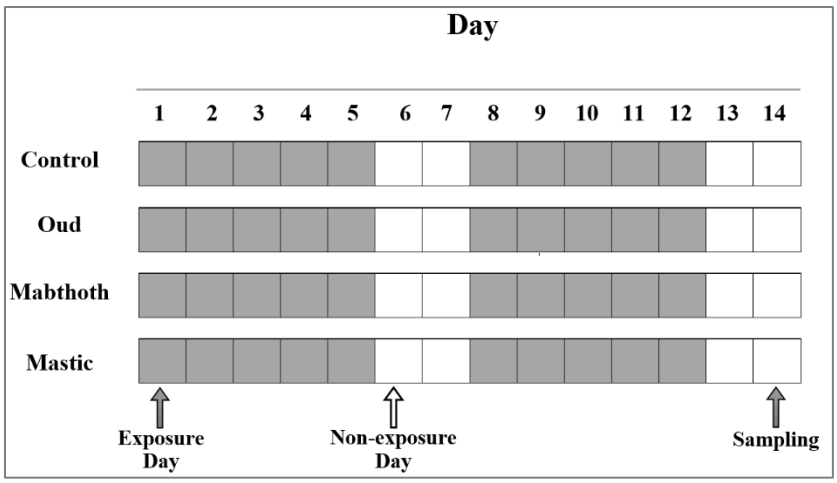

Figure 1 Exposure schedule

2.6. Rats Blood Cell Analysis

2.6.1. Blood Sample Preparation:

After the day of last exposure, blood samples were collected from the orbital sinus. After that, the rats were dissected, and then the spleens were excised (for every three rats from each group). All the rats were sacrificed.

2.6.2. Tests for Immunoglobulin's

Immunoglobulin G (IgG)

First set a blank well without any solution, then add 50µl of Sample per well. Add 50µl of HRP-conjugate to each well immediately (not to Blank well). Mix well and then incubate for 60 minutes at 37°C. After incubation, aspirate each well and wash, repeating the process four times for a total of five washes.

Following that wash by filling each well with Wash Buffer (200µl) using a multi-channel pipette. And let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating. Invert the plate and blot it against clean paper towels.

Add 90µl of TMB Substrate to each well. Incubate for 20 minutes at 37 °C, and protect from light. Add 50µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm.

Immunoglobulin M (IgM)

Add 100µL each standard, blank and sample into the appropriate wells. Then cover the plate with the sealer provided in the kit. Incubate for 90 min at 37°C. Decant the liquid from each well do not wash. Immediately add 100 µL of Biotinylated Detection Ab working solution to each well. Cover the plate with a new sealer. Incubate for 1 hour at 37°C.

Third step decant the solution from each well, add 350μL of wash buffer to each well. Soak for 1-2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.

Add 100μL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 min at 37°C. Decant the solution from each well, repeat the wash process for 5 times as conducted in step 3.

Add 90μL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Preheat the Microplate Reader for about 15 min before OD measurement.

Add 50μL of Stop Solution to each well. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

2.7. Experiments on Rat Spleens

2.7.1. Preparation of Tissue Cell Suspensions

Take tissue slices which have been washed in 0.01M PBS and then a tissue protein extraction reagent has been added according to proportion of 1g to 5-10mL and then mixed on ice bath. After sufficient homogenization, please then centrifuge for 10min at 5000-10000rpm. Take supernatant for immediate testing, or place samples at -20°C/-80°C (1-3 months) for storage.

2.7.2. Measurement of Reactive Oxygen Species (ROS)

Fill the appropriate well of the antibody-precoated microtiter plate with 100 μL of Standards or Samples. To every well, add 50 μL of conjugate. Blend thoroughly. At 37°C, cover and incubate the plate for one hour.

Aspirate the contents of the plate into a sink or suitable waste container to remove the incubation mixture. Once the diluted wash solution has filled each well to the brim, aspirate the contents of the plate into a sink or suitable waste container. For a total of five washes, repeat this process five times. After washing, flip the plate over and blot dry by tapping it against paper towels or absorbent paper until there is no more moisture visible.

Next, fill each well with 50 μL of Substrate A and 50 μL of Substrate B. At 20–25°C, cover and incubate for 15 minutes. Stay out of the sun. Fill each well with 50 μL of Stop Solution then blend thoroughly. Using a microtiter plate reader, quickly read the optical density (O.D.) at 450 nm.

2.7.3. Measurement of Glutathione (GSH)

Fill the matching wells with standards or samples (100μL per well). After using the adhesive tape strip to seal the wells or plate, incubate for 90 minutes at 37 °C. Rinse the ELISA plate twice.

Pour 100μL of the ready-made biotinylated antibody into each well. Use an adhesive tape strip to seal the reaction wells, then incubate for 60 minutes at 37 °C.

Triple-wash the ELISA plate. Place one hundred microliters of the ready enzyme conjugate in each well aside from the blank ones, then wash the ELISA plate 5 times.

Pour 100μL of the ready Color Reagent into each well (as well as the blank well), and then incubate at 37°C with protection from light. The incubation process can be terminated when the coloration of the highest standards becomes darker and a color gradient forms. Add 100μL of Color Reagent C into each well, including the blank well. Blend thoroughly, in ten minutes read the OD at 450 nm.

2.7.4. Measurement of Malondialdehyde (MDA)

MDA levels were calculated in compliance with the kit's guidelines. Fill the matching wells with standards or samples (100μL per well). After using the adhesive tape strip to seal the wells or plate, incubate for 90 minutes at 37°C.

Rinse the ELISA plate twice. Pour 100μL of the ready-made biotinylated antibody into each well. Use an adhesive tape strip to seal the reaction wells, then incubate for 60 minutes at 37°C. Triple-wash the ELISA plate.

Place one hundred microliters of the ready enzyme conjugate in each well aside from the blank ones. ELISA plates should be washed five times. Add 100µL of the ready Color Reagent into each well (as well as the blank well), and then incubate at 37°C with protection from light.

The incubation process can be terminated when the coloration of the highest standards becomes darker and a color gradient form. Add 100µL of Color Reagent C into each well, including the blank well. Blend thoroughly. In ten minutes, read the OD at 450 nm.

2.7.5. Splenic Cell Apoptosis

A spectrophotometer is used to measure the color intensity at 450 nm, where the color shifts from blue to yellow due to the presence of the Stop Solution.

As directed by the manufacturer, an Annexin-V Apoptosis Detection Kit from Pharmingen (Becton Dickinson Company) was employed. Apoptotic cell death, or programmed cell death, is a key process controlling lymphocyte maturation, activation, and development. Furthermore, apoptosis may potentially stop lymphocyte responses from occurring. To the standard well, add 50µl.

Add a 10-l testing sample. The testing sample well receives 40 µl of sample diluent; the blank well receives no additions. Fill each well with 100µl of the HRP-conjugate reagent, cover with an adhesive strip, and incubate at 37°C for 60 minutes. For a total of five washes, aspirate each well and repeat the procedure four times. To each well, add 50µl of each chromogen solution (A and B). Mix gently and let stand at 37°C for 15 minutes. Keep out of the light.

Fill each well with 50µl of Stop Solution. The wells' hue ought ideally shift from blue to yellow. To make sure there is adequate mixing, lightly tap the plate if the color in the wells is green or if the color change is not uniform. In less than 15 minutes, use a microtiter plate reader to read the optical density (O.D.) at 450 nm.

2.7.6. Spleen Lymphocyte Proliferation

The manual differential was used to calculate the lymphocyte cells. Mix 10µl of Leishman's stain with 200µl of spleen suspension, to gain color and appear under a microscope. Then, 10µl from sample was taken and filled to a clean hemocytometer slide chamber with cover slip. The slide was viewed under an inverted phase contrast microscope.

2.8. Histology

After completion of the study, the rats were anesthetized by diethyl ether, the spleen were removed and submitted for histological studies. Spleen were excised and fixed in 10% formalin for a minimum period of 24 h, then transferred to ethanol, embedded in paraffin, sectioned, and stained with hematoxylin-eosin by (Tissue-Tek Prisma®, Sakura Finetek U.S.A.). Slides were scanned at x400 magnification and processed employing the software Nis-Elements F (Nikon).

2.9. Systematic Toxicity Testing

2.9.1. Body Weight Measurement

A change in body weight is an important non-specific indicator in animal studies, which can reflect, an organism's overall health and physiological state. The rats were weighed every morning during the exposure period, using an electronic scale (Adam ACB Plus Portable Balance 43192) and their weight was recorded.

2.10. Statistical Analyses

All of the values are reported as mean +/- standard deviation (SD). Data were analyzed using Statistical Package for Social Sciences (SPSS Inc., version 22, Chicago, IL, USA). The data were analyzed using One-Way ANOVA test followed by Tukey test for comparison between different studied groups. A P <0.05 was statistically significant

3. Results

The impact of exposure to smoke resulting from burning Bakhour on immunological parameters and oxidative stress markers levels in rats were shown below.

3.1. The Levels of IgM in Serum

IgM levels were significantly increased in Mabthoth group versus control, Oud and Mastic groups. Percentage changes of IgM level versus control were increased 177.55% in Oud group, 226.53% in Mabthoth group and 104.08% in Mastic group (Table 1 and Figure 2a).

3.2. The Levels of IgG in Serum

As shown in Table 1, Percentage changes of IgG level versus control were increased 93.38% in Oud group, 127% in Mabthoth group and 61.58% in Mastic group. IgG levels was significantly increased in Mabthoth group versus control, Oud and Mastic groups (Figure 2b).

Table 1 Immunological Parameters IgM and IgG. G1 (Control, Exposure to the fresh air); G2 (Exposure to the smoke of burning Oud); G3 (Exposure to the smoke of burning Mabthoth); G4 (Exposure to the smoke of burning Mastic)

PARAMETERS	(G1)	(G2)	(G3)	(G4)
IgM (g/L)	19.6	54.4	64	40
IgG (mg/L)	108.8	210.4	247	175.8

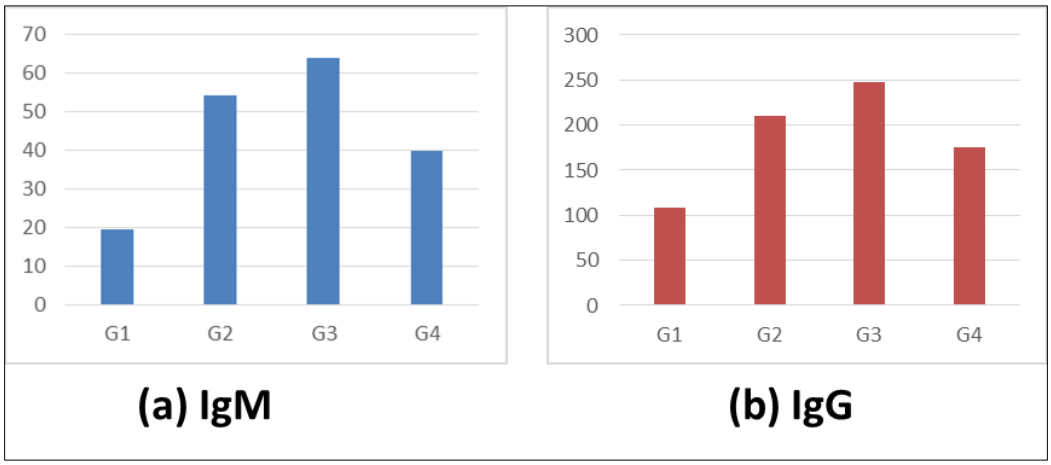

Figure 2 Immunological Parameters IgM and IgG. G1 (Control, Exposure to the fresh air); G2 (Exposure to the smoke of burning Oud); G3 (Exposure to the smoke of burning Mabthoth); G4 (Exposure to the smoke of burning Mastic)

3.3. ROS Level of Splenic Cells

ROS levels were significantly increased in Mabthoth group versus control, Oud and Mastic groups. Percentage changes of ROS levels versus control were increased 395.09% in Oud group, 584.05% in Mabthoth group and 353.99% in Mastic group (Table 2 and Figure 3a).

3.4. GSH Level of Splenic Cells

GSH levels were significantly decreased in Mastic group versus control, Oud and Mabthoth groups. Percentage changes of GSH level versus control were decreased -74.62% in Oud group, -73.64% in Mabthoth group and -82.31% in Mastic group (Table 2 and Figure 3b).

3.5. MDA Level of Splenic Cells

MDA levels were significantly increased in Mabthoth group versus control, Oud and Mastic groups. Percentage changes of MDA levels versus control were increased 245.65% in Oud group, 284.78% in Mabthoth group and 263.04% in Mastic group (Table 2 and Figure 3c).

3.6. Apoptosis of Splenic Cells

P53 Apoptosis levels were significantly increased in Mabthoth group versus control, Oud and Mastic groups. Percentage changes of P53 Apoptosis levels versus control were increased 1140.91% in Oud group, 1295.45% in Mabthoth group and 601.136% in Mastic group (Table 2 and Figure 3d).

3.7. Splenic Lymphocyte Proliferation

Lymphocytes rate were significantly increased in Mabthoth group versus control, Oud and Mastic groups. Percentage changes of Lymphocytes rate versus control were increased 244.61% in Oud group, 278.32% in Mabthoth group and 71.91% in Mastic group (Table 4.2 and Figure 4.2e).

Table 2 Oxidative Stress Markers and Apoptosis Levels. G1 (Control, Exposure to the fresh air); G2 (Exposure to the smoke of burning Oud); G3 (Exposure to the smoke of burning Mabthoth); G4 (Exposure to the smoke of burning Mastic)

Parameters	**(G1)**	**(G2)**	**(G3)**	**(G4)**
ROS (nmol/min/ mg protein)	1.68	8.58	11.49	7.39
GSH (nmol/gram protein)	13.82	3.78	3.36	2.58
MDA (nmol/gram protein)	0.50	1.52	1.73	1.69
P53 Apoptosis (U/ml)	0.94	10.61	12.04	6.50
Lymphocytes (%)	8.48	32.20	32.40	15.78

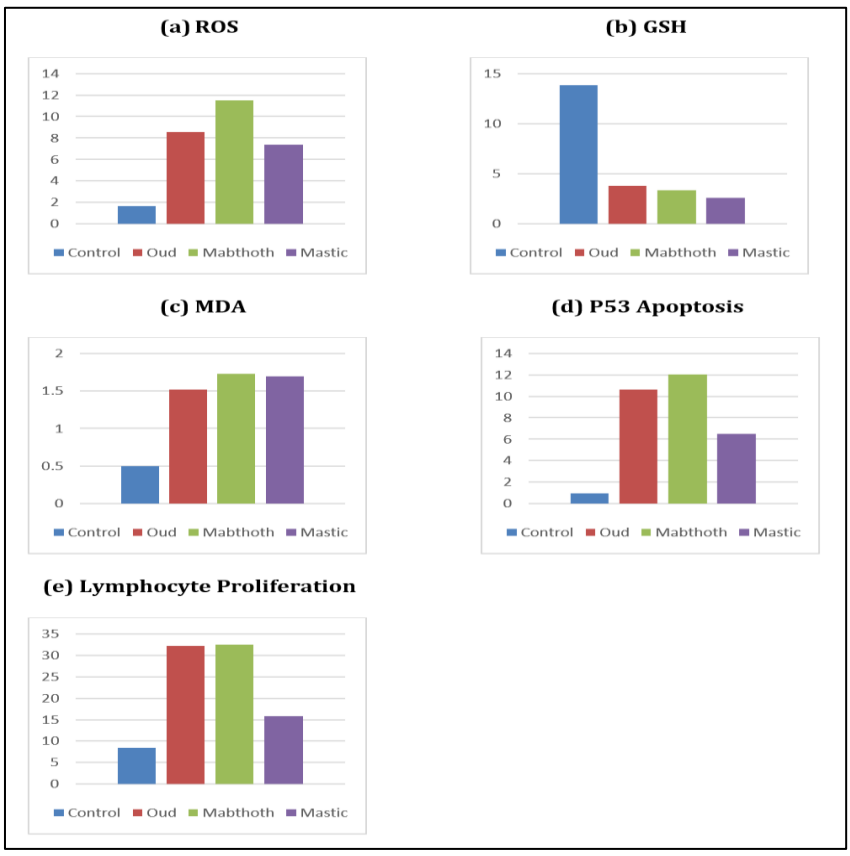

Figure 3 Experimental results of rat spleens testing. (a) ROS level ; (b) GSH level ; (c) MDA level ; d) P53 Apoptosis and (e) Levels of the ability of spleen lymphocyte proliferation in rat. G1 (Control, Exposure to the fresh air); G2 (Exposure to the smoke of burning Oud); G3 (Exposure to the smoke of burning Mabthoth); G4 (Exposure to the smoke of burning Mastic)

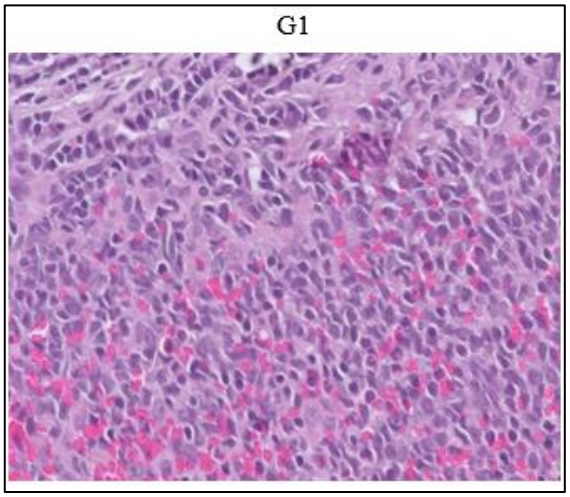

Figure 4 Photomicrographs of a section in the spleen of control rat showing normal histology of both red and white pulp (H&E ×400)

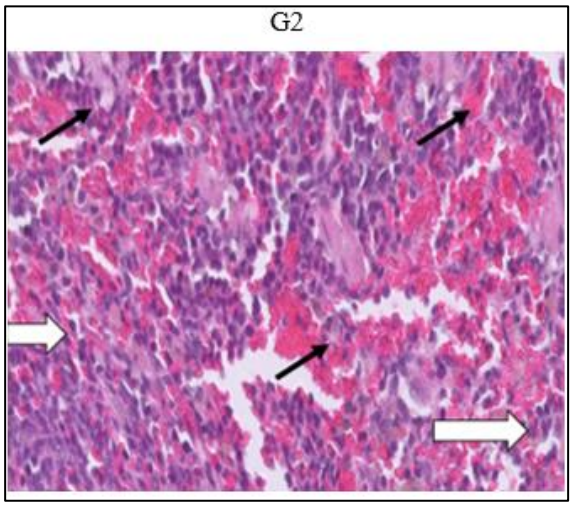

Figure 5 Photomicrographs of a section in the spleen of rat exposure to the smoke of burning Oud showing severe splenic hemorrhage (black arrows) with hemosiderin pigment deposition in the red pulp; lymphoid cell depletion with presence of many tangible body macrophages (white arrows) detected within the splenic periarteriolar lymphoid follicles mononuclear inflammatory cell infiltrations (white arrow) (H&E ×400)

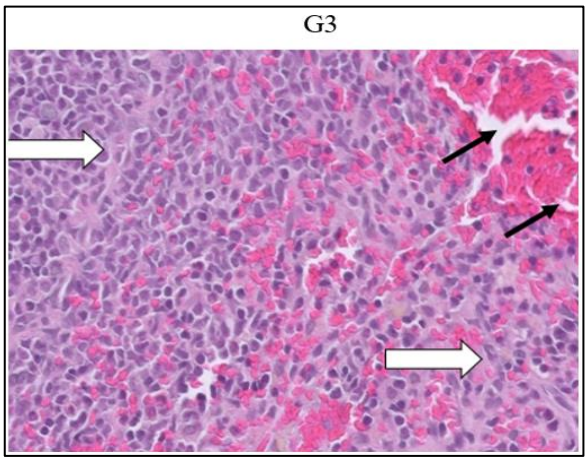

Figure 6 Photomicrographs of a section in the spleen of rat exposure to the smoke of burning Mabthoth showing moderate of splenic hemorrhage (black arrows) with hemosiderin pigment deposition in the red pulp; lymphoid cell depletion with presence of many tangible body macrophages (white arrows) detected within the splenic periarteriolar lymphoid follicles mononuclear inflammatory cell infiltrations (white arrow) (H&E ×400)

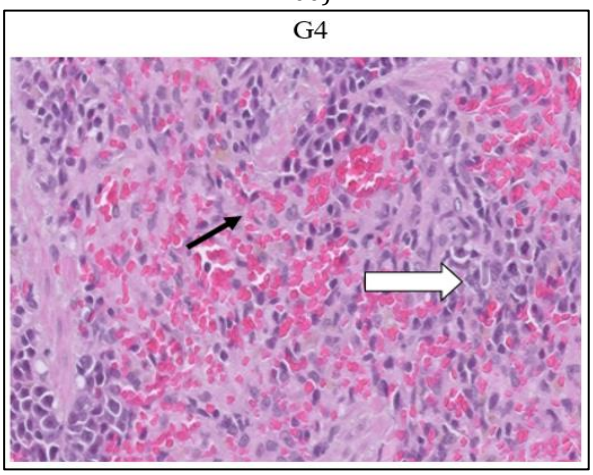

Figure 7 Photomicrographs of a section in the spleen of rat exposure to the smoke of burning Mastic showing mild of splenic hemorrhage (black arrows) with hemosiderin pigment deposition in the red pulp; lymphoid cell depletion with presence of many tangible body macrophages (white arrows) detected within the splenic periarteriolar lymphoid follicles mononuclear inflammatory cell infiltrations (white arrow) (H&E ×400)

3.8. Histology

Qualitative histological analysis of spleen showed many results could see in the figures 4-7.

3.9. Changes in Body Weights after Exposure

The rat (5 rat/group) were exposed to Bakhour smoke for 2 weeks. During the 2 weeks of exposure, a trend for increasing body weight was noted in each group, but there were no significant differences in body weight between the four groups (Table 3 and Figure 8).

Table 3 Body Weight of the Experiment Rat (g) During 3 Weeks. G1 (Control, Exposure to the fresh air); G2 (Exposure to the smoke of burning Oud); G3 (Exposure to the smoke of burning Mabthoth); G4 (Exposure to the smoke of burning Mastic)

Weeks	***(G1)***	***(G2)***	***(G3)***	***(G4)***
Week 1	184.4	173.4	195	164.4
Week 2	191	174	196.4	166
Week 3	191.4	175.6	197	166.4

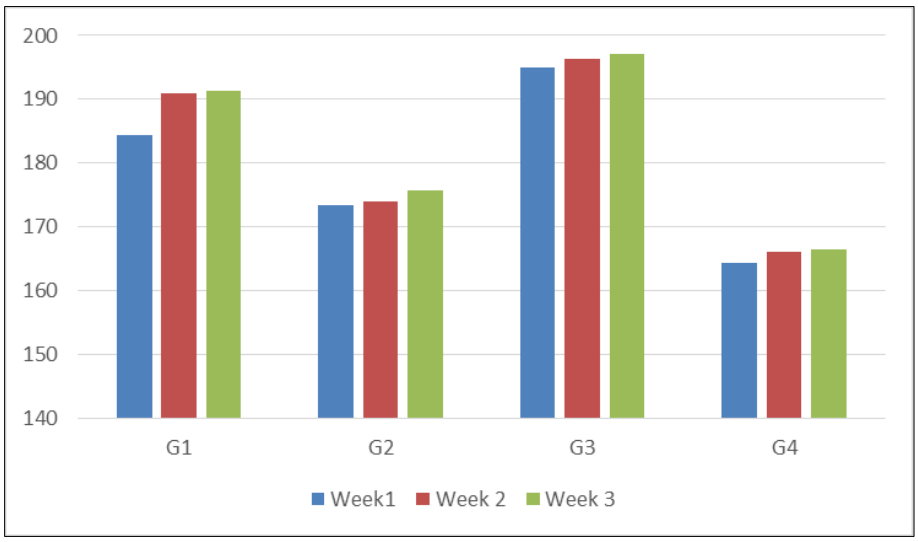

Figure 8 Average of Body Weights (g). G1 (Control, Exposure to the fresh air); G2 (Exposure to the smoke of burning Oud); G3 (Exposure to the smoke of burning Mabthoth); G4 (Exposure to the smoke of burning Mastic)

3.10. Statistical Analyses

All of the values are reported as mean +/- standard deviation (SD). Data were analyzed using Statistical Package for Social Sciences (SPSS Inc., version 22, Chicago, IL, USA). The data were analyzed using One-Way ANOVA test followed by Tukey test for comparison between different studied groups. A P <0.05 was statistically significant.

4. Discussion

In spite of regulatory controls, the broad masses of the people are exposed to hazardous air pollutants (HAPs) with potential to cause cancer or other serious health effects. Volatile organic compounds (VOCs) are one of prominent pollutants in indoor air. Moreover, Inhalation of them may cause immune system and hematological parameter changes [7].

Previous studies reported increased levels of oxidative stress as a result of exposure to household air pollution, These results are consistent with our animal experiment results [8].

The widespread use of incense in the Arabian tradition for several domestic purposes has prompted the need for investigation on whether incense smoke exposure might carry hazards for human health [9]. Burning coal to produce the Bakhour smoke had been shown to lead to a rise in the amount of sulfur dioxide in the air, which could cause severe respiratory problems [2].

The spleen is the largest immune organ in the body and plays an important role in the immune response [7]. However, there have been no reports of research on oxidative damage to the spleen caused by exposure to incense smoke. We have measured the ROS, MDA, GSH levels, cell apoptosis and lymphocytes in rat after exposed to Bakhour smoke.

A number of previous studies had reported links between incense smoke and various health hazards that affect the eyes, nose, skin, and throat. Several studies had also previously linked the fumes from burning incense to respiratory problems, allergies, and asthma [2].

Furthermore, a number of studies on incense smoke showed that the fumes contain a significant amount of PM, gases such as CO, CO2, NO2, SO2, among others, and a range of volatile organic compounds, such as benzene, toluene, xylenes, aldehydes and PAHs [10]. Since the benzene compound is one of the results of the burning of Bakhour, the previous studies were discussed that linked the exposure to benzene and its impact on immunity.

Previous studies found a significant correlation between the concentration of benzene in the blood and immune suppression. Benzene and its metabolites induce oxidative stress through excessive production of ROS and MDA and decrease in GSH it also causes immune dysfunction and subsequently alteration in p53 expression [11].

The combustion of Bakhour, wood, cigarette and the burning of candles are an important source of residential indoor particulate matter, especially in the 2.5 mm size range and below. Interestingly, Bakhour burning produces over 4 times more particulate matter than cigarette smoke [9]. Previous studies have shown that e-cigarettes are associated with an increase in oxidative stress such as malondialdehyde and ROS [12,13], it also e-cig exposed mice contained significantly elevated levels of oxidative stress [14].

In the present study, animals exposed to the smoke of Bakhour revealed significant ultrastructure changes in their spleen tissue. Splenic hemorrhage is showen with hemosiderin pigment deposition in the red pulp.

It was also noted lymphoid cell depletion with presence of many tangible body macrophages. Detected within the splenic periarteriolar lymphoid follicles mononuclear inflammatory cell infiltrations. In addition, as previous study splenic was observed in mice exposer to benzene. Microscopically, the atrophy of white pulp and the loss of small lymphocytes in lymphoid nodules were found in spleen [15]. The present data can be considered as an evidence that inhalation of Bakhour smoke, which is a common local tradition, can induce changes in spleen tissue as evidenced by electron microscopy [16].

It has been found that inhaling incense smoke may cause changes in the activity of GSH levels in various tissues and organs. The results of spleen samples and blood indicated that Bakhour smoke exposure is associated with increased lipid peroxidation which in turn augments further production of free radicals [7].

A significant decrease in the level of GSH was observed 74.62% in Oud group, 73.64% in Mabthoth group and 82.31% in Mastic group compared to the control group. Our results are consistent with previous studies that have shown that exposure of rat to Incense smoke induced a significant decrease in GSH while it revealed a significant increase in MDA and increased the ROS production of rat splenocytes, the same as that reported in our finding [11,17,18].

In this study, exposure to Bakhour smoke increased MDA levels in spleen tissue. The Mabthoth smoke exposure exhibited significantly higher effects on spleen MDA levels than Oud. The higher MDA level was observed 284.78% in Mabthoth group, 245.65% in Oud group and 263.04% in Mastic group than controls. As previous studies rats exposed to incense also displayed a derangement of oxidative status compared to the control group, with a significant reduction in GSH and a significant increment in MDA [19].

All of groups increased ROS in the spleen tissue, while reducing the antioxidant capacity (GSH level). Previous studies reported increase in ROS level compared with controls [11].

In the current study a statistically significant increase in ROS level was observed 395.09% in Oud group, 584.05% in Mabthoth group and 353.99% in Mastic group compared with controls.

IgM and IgG levels were significantly increased in Mabthoth group versus control, Oud and Mastic groups in our study. Previous studies they found that incense burning was a risk factor for elevated IgE, they also finding links between incense smoke chemicals and elevated IgE levels [10,20].

Normal function of the p53 is essential in DNA repair, cell cycle control, apoptosis and acts to integrate multiple stress signals into a series of diverse anti-proliferative responses. In the present study, the expression of p53 was significantly higher in the study groups exposed to Bakhour smoke. Percentage changes of P53 Apoptosis levels versus control were increased 1140.91% in Oud group, 1295.45% in Mabthoth group and 601.136% in Mastic group.

The apoptotic effects (caspase-3 increased) we observed in the spleen tissues in the present study probably result from the oxidative stress caused by the exposure of Bakhour smoke [7].

The results of the spleen lymphocyte proliferation experiment showed that the ability of lymphocytes to proliferate significantly increase, which meant that exposure to Bakhour smoke had a Stimulating effect on the ability of lymphocytes to proliferate.

Lymphocytes rats were significantly increased in Mabthoth group versus control, Oud and Mastic groups. Percentage changes of Lymphocytes rats versus control were increased 244.61% in Oud group, 278.32% in Mabthoth group and 71.91% in Mastic group.

A change in body weight is an important non-specific indicator in animal studies, which can reflect, an organism's overall health and physiological state. In this study, the weight gain curves of rat were seen to fit closely together [7].

Opened windows and doors or distancing oneself from Bakhour burners/censers would, therefore, effectively reduce the risk of smoke exposure. Hence, the duration or frequency of incense burning is likely to be a factor influencing. The small sample size in this study may be another reason for our findings. Further studies are needed in this regard.

5. Conclusion and Recommendations

This is one of the first studies to demonstrate the effect of Bakhour smoke on immune system in rats. The finding of adverse effects of Bakhour smoke in animal system made here have considerable relevance to humans considering the widespread use of Bakhour. Burning of Bakhour and incense sticks is a common practice across the whole globe, Meanwhile there is a major gap of knowledge with regard to the contents of the smoke emitted into the environment.

In this study, the experiments of rats were fund Immunological Parameters and Oxidative Stress Markers were significantly increased except GSH is decreased versus control, which led to immunosuppression. And it also causes immune dysfunction and subsequently alteration in p53 expression. As Bakhour use is very common in most Arab communities, our findings have important public health implications. With the currently available evidence, it is important to raise public awareness about the potential harmful effects of Bakhour burning in order to take steps to reduce exposure such as reducing the frequency and duration of Bakhour burning, keeping the room well ventilated when burning bakhour and avoiding such practice in the presence of children and susceptible individuals. It will effectively dilute the indoor air pollutants and hence reduce the risk of exposure.

For future studies, a longitudinal study design with a larger sample size is recommended for examining the influence of burning Bakhour on immune system. It is also possible to study other types of Bakhour and experiment with the effect of Bakhour burned using charcoal. Bakhour smoke samples can also be examined using a gas chromatography device to determine the components of each type.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

All animal experimentation was approved by the Medical Scientific Foundation for Research and Development (MSF) of the King Abdulaziz University, Jeddah, Saudi Arabia. (Reference Number: p100-2023).

References

[1] M. Ljaljević Grbić et al., "Frankincense and myrrh essential oils and burn incense fume against micro-inhabitants of sacral ambients. Wisdom of the ancients?," J. Ethnopharmacol., vol. 219, no. February, pp. 1–14, 2018, doi: 10.1016/j.jep.2018.03.003.

[2] S. Dalibalta, Y. Elsayed, F. Alqtaishat, I. Gomes, and N. Fernandes, "A health risk assessment of Arabian incense (Bakhour) smoke in the United Arab Emirates," Sci. Total Environ., vol. 511, pp. 684–691, 2015, doi: 10.1016/j.scitotenv.2014.12.024.

[3] S. Dalibalta, Y. Elsayed, F. Alqtaishat, I. Gomes, and N. Fernandes, "Science of the Total Environment A health risk assessment of Arabian incense (Bakhour) smoke in the United Arab Emirates ☆," Sci. Total Environ., vol. 511, pp. 684–691, 2015, doi: 10.1016/j.scitotenv.2014.12.024.

[4] N. D. Dimitrova, R. Y. Kostadinova, S. N. Marinova, T. A. Popov, and T. I. Panev, "Specific immune responses in workers exposed to benzene," vol. 5, pp. 1554–1559, 2005, doi: 10.1016/j.intimp.2005.04.012.

[5] T. B. Garden, "Article • January 2014," no. January 2014, 2016.

[6] J. Niebler, "Incense Mate 4.," pp. 63–86.

[7] H. Wen et al., "Effects of combined exposure to formaldehyde and benzene on immune cells in the blood and spleen in Balb/c mice," Environ. Toxicol. Pharmacol., vol. 45, pp. 265–273, 2016, doi: 10.1016/j.etap.2016.05.007.

[8] S. Salvi and K. Apte, "Household air pollution and its effects on health," F1000Research, vol. 5, pp. 1–13, 2016, doi: 10.12688/f1000research.7552.1.

[9] M. S. Alokail, N. M. Al-Daghri, S. A. Alarifi, H. M. Draz, T. Hussain, and S. M. Yakout, "Long-term exposure to incense smoke alters metabolism in Wistar albino rats," Cell Biochem. Funct., vol. 29, no. 2, pp. 96–101, 2011, doi: 10.1002/cbf.1726.

[10] T. C. Lin, G. Krishnaswamy, and D. S. Chi, "Incense smoke: Clinical, structural and molecular effects on airway disease," Clin. Mol. Allergy, vol. 6, pp. 1–9, 2008, doi: 10.1186/1476-7961-6-3.

[11] N. Uzma, S. S. Kumar, and M. A. H. Hazari, "Exposure to benzene induces oxidative stress, alters the immune response and expression of p53 in gasoline filling workers," Am. J. Ind. Med., vol. 53, no. 12, pp. 1264–1270, 2010, doi: 10.1002/ajim.20901.

[12] T. Marczylo, "How bad are e-cigarettes? What can we learn from animal exposure models?," J. Physiol., vol. 598, no. 22, pp. 5073–5089, 2020, doi: 10.1113/JP278366.

[13] H. Qasim et al., "Short-term E-cigarette exposure increases the risk of thrombogenesis and enhances platelet function in mice," J. Am. Heart Assoc., vol. 7, no. 15, pp. 13–15, 2018, doi: 10.1161/JAHA.118.009264.

[14] T. E. Sussan et al., "Exposure to electronic cigarettes impairs pulmonary anti-bacterial and anti-viral defenses in a mouse model," PLoS One, vol. 10, no. 2, pp. 1–15, 2015, doi: 10.1371/journal.pone.0116861.

[15] B. Inhalation, L. Subpopulations, and I. Response, "Inhalation," pp. 92–101, 1986.

[16] S. A. Alarifi, M. M. Mubarak, and M. S. Alokail, "Ultrastructural changes of pneumocytes of rat exposed to Arabian incense (Bakhour)," Saudi Med. J., vol. 25, no. 11, pp. 1689–1693, 2004.

[17] A. Skovmand, A. C. Damiao Gouveia, I. K. Koponen, P. Møller, S. Loft, and M. Roursgaard, "Lung inflammation and genotoxicity in mice lungs after pulmonary exposure to candle light combustion particles," Toxicol. Lett., vol. 276, pp. 31–38, 2017, doi: 10.1016/j.toxlet.2017.04.015.

[18] S. Nady et al., "Study on the Biochemical Effect of Date Palm Pollen on Mice Exposed to Incense Smoke," Cairo Univ, vol. 82, no. 1, pp. 495–504, 2014, [Online]. Available: www.medicaljournalofcairouniversity.net.

[19] V. K. Yadav et al., "Health and Environmental Risks of Incense Smoke: Mechanistic Insights and Cumulative Evidence," J. Inflamm. Res., vol. 15, pp. 2665–2693, 2022, doi: 10.2147/JIR.S347489.

[20] M. Jareebi et al., "Exploring the Relationship Between Bakhour Use and Respiratory Health: A Cross-Sectional Study in the Jazan Region, Saudi Arabia," Int. J. Gen. Med., vol. Volume 17, pp. 2833–2845, 2024, doi: 10.2147/ijgm.s461772.