Incense smoke inhalation affects spermatogenesis and sperm quality and impairs liver and kidney function in adult male rats

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ABSTRACT

Background: Despite the growing evidence indicating the association between inhalation of traditional incense smoke (IS) and the increased risk of numerous health issues, its possible effect on spermatogenesis is still confusing. Aim: The present study was designed to address the hypothesis that exposure to traditional IS may impact sperm quality and affect liver and kidney function. Material and Methods: Using a rat model, we evaluated the effects of IS exposure on semen quality by Computer-Assisted Semen Analysis (CASA) and blood samples were collected for the determination of random blood glucose, C-reactive protein (CRP), liver and kidney function parameters and testosterone levels. Results: Prolonged inhalation of IS caused a highly significant decrease in testosterone levels compared with the control group. Moreover, complete semen analysis indicated that sperm’s progressive motility, velocity, and vigor parameters were significantly decreased in IS-exposed groups compared with the unexposed group. Also, IS inhalation significantly increased the percentage of morphologically abnormal sperms. Biochemically, prolonged inhalation of IS caused a significant decrease in blood glucose while blood CRP, liver, and kidney function biomarkers were significantly elevated compared with the control group, reflecting potential toxicity. Conclusion: We concluded that excessive and prolonged IS inhalation may cause a remarkable deterioration in spermatogenesis, sperm quality as well as kidney and liver function.

Keywords: Incense smoke; Liver and kidney functions; Spermatogenesis

INTRODUCTION

Incense (from Latin incendere “to burn”) is made of aromatic biological materials and essential oils when burn emitting fragrant smoke (Qin et al., 2019). There are different forms of incense available commercially including sticks, cones, coils, rope, and smudge bundles. It has been hypothesized that incense burning is a source of indoor air pollution that harms those who are exposed to it (Cohen et al., 2013). Most types of incense sticks share the same structure and various reports showed that in general during their burning process, they generate smoke consisting of a gaseous phase of carbon dioxide, carbon monoxide, sulfur dioxide, formaldehyde, nitrogen dioxide, polycyclic aromatic compounds, volatile organic compounds (VOCs), toxic heavy metals and particulate matter (PM) (Friborg et al., 2008; Ji et al., 2010; Geng et al., 2019; Yadav et al., 2020; Yadav et al., 2021).

Several studies have reported elevated levels of these toxic organic compounds in IS and suggested a negative association with sperm quality and the spermatogenesis process in human and animal models (Xiao et al., 2001; Yang et al., 2007; Zhang et al., 2015; Mahgoub & Salih, 2017; Lv et al., 2022). Burning incense at home is a long-standing and widespread practice among the people of Asian nations and the Middle East; these areas have higher percentages of male infertility worldwide. A minimum of 30 million men are diagnosed as infertile worldwide, with the highest rates detected in Africa (Agarwal et al., 2015). Male infertility is generally owed to the decline in counts and quality of spermatozoa which were reported to be highly disrupted by oxidative stress resulting from the generation of reactive
oxygen species (ROS) targeting spermatozoa plasma membrane and DNA (Alahmar, 2019). Exposure to environmental pollutants is being recognized as a potential cause of infertility and despite its worldwide increasing incidence, limited reports focused on exploring novel lifestyle-associated environmental risk factors that might contribute to idiopathic male infertility. (Gabrielsen & Tanrikut, 2016; Vecoli et al., 2016; Jurewicz et al., 2018; Mima et al., 2018) Growing evidence suggests an association between exposure to IS and the increased risk of numerous human health issues (Kim et al., 2014; Kim et al., 2019; Akingbade et al., 2021; Lee et al., 2021). Recently, it was demonstrated that Carlina gummifera IS inhalation caused oxidative stress in the testis, which may be the indirect cause of the observed changes in spermatogenesis (Dorsaf et al., 2022). Therefore, the present study was designed to investigate the hypothesis that prolonged IS inhalation may damage sperm quality, liver and kidney normal functions in adult male rats subjected to low and high doses of IS.

**MATERIAL AND METHODS**

**Animals**

This study was conducted on forty-five sexually mature Sprague-Dawley male rats, aged 70-80 days and weighing 130 – 150 g. Rats were purchased and housed in the experimental animal house, Faculty of Pharmacy, Pharos University, Alexandria, Egypt. Rats were housed in metal cages (15 rats/cage) and maintained at approximately 23-25°C with a 12:12h light/dark cycle and received laboratory basal diet and tap water for a one-week acclimation period and throughout the whole study. All animal procedures and experimental protocols were approved by the Research Ethics Committee of the medical research institute, Alexandria University (AU012282233). All animal experiments were performed according to the ARRIVE guidelines and the National Research Council’s guide for the care and use of laboratory animals.

**Materials**

The most common incense sticks in Egypt were used in the current study (Ansam®, 0.4 g/Makkah incense stick, SAK).

**Animal Treatments**

Rats were randomly divided into three groups (15 rats/ group). Group I included 15 rats unexposed to IS and served as the control group. Group II included 15 rats exposed to a low dose of IS (1.6 g) for 30 min daily for four weeks. Group III included 15 rats exposed to a high dose of IS (3.2 g) for 30 min daily for four weeks. Rats were exposed to incense smoke in an inhalation chamber with dimensions of 55 cm by length, 40 cm by width, and 20 cm by height as shown in Figure 1. The ratio of chamber volume (44-L) to the mean body weight of rats (140 g) was 314, which is in proportionate to that of the ratio (317) of standard male body weight (85 kg) in a standard living room volume (27,000 L) (Yamamoto et al., 2021).

**Blood sample collection**

Rats were sacrificed at the end of the experiment and blood samples were taken through heart puncture. The blood sample was collected in a non-heparinized (plain) tube, left to clot for 15 min and the serum was separated by centrifugation then the serum was separated and stored at -20°C for further use.

**Determination of Biochemical Parameters**

Serum levels of glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, and creatinine were determined according to manufacturer’s protocols (Bio-diagnostic Co., Egypt) using double beam spectrophotometer (Shimadzu, Kyoto Japan). Serum CRP and testosterone levels were determined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s protocols (DRG Diagnostics, Marburg, Germany).

![Figure 1. Incense smoke inhalation chamber](image-url)
Semen Analysis

To allow spermatozoa release, freshly dissected portions of the epididymis cauda were cut three times and placed in a petri dish containing 500 µL tris-citric acid-fructose (Tris 3.025 g, citric acid 1.7 g, fructose 1.25 g, distilled water 100 mL) and warmed for 5 minutes at 37°C. This fluid was collected in aliquots for semen analysis (Lima et al., 2018).

Computer-assisted semen analysis (CASA) with a phase contrast microscope was used to assess sperm motility immediately after collection. The equipment was calibrated for rodent spermatozoa, and motility parameters including the spermatozoa motility (motility, %), the progressive motility (PROG, %), the velocity average path (VAP, m/s), the velocity curved line (VCL, m/s), the velocity straight line (VSL, m/s), wobble (WOB, %), straightness (STR, %), linearity (LIN, %), beat cross frequency (BCF, Hz) and amplitude of lateral head displacement (ALH, m) were assessed.

For sperm morphology analysis, 50 µL of epididymal fluid was fixed in 100 µL of buffered formaldehyde (4%). 200 cells from each rat were examined under phase-contrast microscopy, and categorized as normal or abnormal according to the strict sperm morphology criteria. The morphological abnormalities were divided into head, neck, and tail defects. The percentages of normal and abnormal-shaped sperms were determined.

Statistical analysis

Data were examined and analyzed using IBM SPSS software package version 20.0. The Kolmogorov-Smirnov test was used to verify the normality of the distribution of quantitative variables. Quantitative data were described using range (minimum and maximum) and mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used for normally distributed quantitative variables to compare between more than two groups, and the Post Hoc test (Bonferroni) for pairwise comparisons. The significance of the obtained results was judged at the 5% level. *P value ≤ 0.05, **P ≤ 0.01, and ***P ≤ 0.001 were considered significant, very significant, and highly significant, respectively.

RESULTS

Effect of IS on testosterone levels

The range and mean ± SD for testosterone levels (nmol/L) are shown in Table 1. Inhalation of IS has caused a significant decrease in testosterone levels in low-dose and high-dose groups compared to the control unexposed group (0.28 ± 0.03, 0.3 ± 0.1, and 3.9 ± 0.2, respectively).

Effect of IS on sperm quality

As presented in Table 2, the motile spermatozoa (MOT, %), in low and high-dose groups were significantly higher than that in the control unexposed group (P= 0.008, p= 0.001; respectively). However, sperm progressive motility (PROG, %) in low and high dose groups was lower in low and high dose groups compared to the unexposed group (12.8 ± 0.4, 12.8 ± 1.2, and 15.3 ± 0.5, respectively). Moreover, the mean sperm velocity average path (VAP, m/s), velocity curved line (VCL, m/s) and velocity straight line VSL (m/s) in IS exposed groups were significantly lower than the control group. Additionally, the mean wobble (WOB, %) in low-dose and high-dose groups were significantly lower than that in the unexposed group (34.3 ± 2.99, 39.2 ± 5, and 50 ± 3.8, respectively). On the other hand, statistically insignificant decreases in straightness (STR, %), linearity (LIN, %), the amplitude of lateral head displacement (ALH, m) and beat cross frequency (BCF, Hz) were observed in IS-exposed groups compared to the controls. Sperm morphology analysis is shown in Table 3, the sperm deformity index (SDI) value in low-dose and high-dose groups was significantly higher than that in the unexposed group (9.6 ± 2.4, 91 ± 2.5, and 85.8 ± 0.1, respectively). Finally, the terato sperm percentage (sperms with abnormal morphology) in low-dose and high-dose groups was significantly higher than that in the unexposed group (25.6 ± 2.4, 91 ± 2.5, and 85.8 ± 0.1, respectively). The predominant morphological defects were tapered or big heads, small acrosome heads, bent or thick necks, asymmetric insertion necks, bent, broken, or coiled tails.
Figure 2. Microphotographs illustrating sperm morphology in the unexposed group (IA & IB), low-dose group (IIA-IID), and high-dose group (IIIA & IIIB).
Incense smoke inhalation affects spermatogenesis and sperm quality and impairs liver and kidney function in adult male rats.

**Table 1. Testosterone levels in studied groups.**

<table>
<thead>
<tr>
<th>Testosterone level (nmol/L)</th>
<th>Group I (n = 15)</th>
<th>Group II (n = 15)</th>
<th>Group III (n = 15)</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>3.6 – 4.2</td>
<td>0.2 – 0.3</td>
<td>0.17 – 0.4</td>
<td>1561.691*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>3.9 ± 0.2</td>
<td>0.28 ± 0.03</td>
<td>0.3 ± 0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Significance:**
- p1<0.001**
- p2<0.001***
- p3=0.857

Group I: unexposed, Group II: low-dose group, and Group III: high-dose group. p1: p value for comparing between group I and group II; p2: p value for comparing between group I and group III, p3: p value for comparing between group II and group III. **: Statistically significant at p ≤ 0.05. ***: Statistically significant at p ≤ 0.001.

**Table 2. Sperm motility analysis**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (n = 15)</th>
<th>Group II (n = 15)</th>
<th>Group III (n = 15)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOT (%)</td>
<td>25.8 – 26.3</td>
<td>27.99 – 30.3</td>
<td>29.9 - 31.1</td>
<td>26.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>26.04±0.2</td>
<td>29.1 ± 1.2</td>
<td>30.5 ± 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td>P1=0.008**, P2=0.001***, P3=0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PROG (%)</td>
<td>14.8 – 15.8</td>
<td>12.3 – 13.2</td>
<td>11.7 – 14</td>
<td>10.4</td>
<td>0.011</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>15.3 ± 0.5</td>
<td>12.8 ± 0.4</td>
<td>12.8 ± 1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td>P1= 0.02**, P2=0.02, P3=1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAP (m/s)</td>
<td>(3.97 – 5.6)10^-6</td>
<td>(2.3 – 2.9)10^-6</td>
<td>(3.9 – 4.4)10^-6</td>
<td>13.79</td>
<td>0.006</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>(4.8 ± 0.8)10^-6</td>
<td>(2.6 ± 0.3)10^-6</td>
<td>(4.1 ± 0.2)10^-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td>P1= .006**, P2=0.5, P3=0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCL (m/s)</td>
<td>(7.3 – 11.1)10^-6</td>
<td>(5.3 – 6.7)10^-6</td>
<td>(8 – 8.2)10^-6</td>
<td>5.5</td>
<td>0.04</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>(9.3 ± 2)10^-6</td>
<td>(5.98 ± 0.7)10^-6</td>
<td>(8.1 ± 0.1)10^-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td>P1= 0.05**, P2=0.9, P3=0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VSL (m/s)</td>
<td>(1.5 – 1.9)10^-4</td>
<td>(0.9 – 1.2)10^-4</td>
<td>(1.29 – 1.4)10^-4</td>
<td>14</td>
<td>0.005</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>(1.7 ± 0.2)10^-4</td>
<td>(1.1 ± 0.1)10^-4</td>
<td>(1.3 ± 0.1)10^-4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td>P1= 0.006**, P2=0.08, P3=0.2</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>STR (%)</td>
<td>34.3 – 37.7</td>
<td>30.3 – 35.1</td>
<td>23.8 – 33.4</td>
<td>3.9</td>
<td>0.084</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>35.97±1.7</td>
<td>32.7 ± 2.4</td>
<td>28.6 ± 4.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td>P1= 0.8, P2=0.1, P3=0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LIN (%)</td>
<td>15.9 – 20.7</td>
<td>12.7 – 15.5</td>
<td>10.9 – 16.5</td>
<td>3.7</td>
<td>0.088</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>18.3 ± 2.4</td>
<td>14.1 ± 1.4</td>
<td>13.7 ± 2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td>P1= 0.2, P2=0.1, P3=1</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>WOB (%)</td>
<td>46.2 – 53.7</td>
<td>31.3 – 37.3</td>
<td>34.2 – 44.2</td>
<td>11.9</td>
<td>0.008</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>50 ± 3.8</td>
<td>34.3 ± 2.99</td>
<td>39.2 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td>P1= 0.009**, P2=0.05, P3=0.5</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>ALH (m)</td>
<td>(6 – 6.13)10^-4</td>
<td>(4.47 – 5.2)10^-4</td>
<td>(4.46 – 5.96)10^-4</td>
<td>5.05</td>
<td>0.052</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>(6.1 ± 0.06)10^-4</td>
<td>(4.8 ± 0.4)10^-4</td>
<td>(5.2 ± 0.8)10^-4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td>P1= 0.06, P2=0.2, P3=1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCF (Hz)</td>
<td>2.25 – 2.52</td>
<td>2.06 – 2.47</td>
<td>2.09-2.28</td>
<td>1.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.4 ± 0.1</td>
<td>2.3 ± 0.2</td>
<td>2.2 ± 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td>P1= 1, P2=0.4, P3=1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Group I: unexposed, Group II: low-dose group and Group III: high-dose group. p1: p value for comparing between group I and group II; p2: p value for comparing between group I and group III, p3: p value for comparing between group II and group III. **: Statistically significant at p ≤ 0.05. ***: Statistically significant at p ≤ 0.001. (MOT: motility, PROG: progressive motility, VAP: velocity average path, VCL: velocity curved line, VSL: velocity straight line, STR: straightness, LIN: linearity, WOB: wobble, ALH: amplitude of lateral head displacement, BCF: beat cross frequency).

**Table 3. Sperm morphology analysis**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (n = 15)</th>
<th>Group II (n = 15)</th>
<th>Group III (n = 15)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDI</td>
<td>1.57 – 2</td>
<td>3.17 – 3.6</td>
<td>2.3 – 3.25</td>
<td>19.36</td>
<td>0.002</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.8 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>2.79 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td>P1=0.003*, P2=0.02*, P3=0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal sperms (%)</td>
<td>14 – 14.3</td>
<td>7.2 – 11.9</td>
<td>6.5 – 11.6</td>
<td>5.89</td>
<td>0.038</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>14.15 ± 0.1</td>
<td>9.6 ± 2.4</td>
<td>9 ± 2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td>P1= 0.03*, P2=0.02*, P3=0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terato sperms (%)</td>
<td>Range</td>
<td>85.7 – 86</td>
<td>88 – 92.8</td>
<td>88.4 – 93.2</td>
<td>5.89</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>85.8 ± 0.1</td>
<td>90.4 ± 2.4</td>
<td>91 ± 2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td>P1=0.03*, P2=0.02*, P3=0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Group I: unexposed, Group II: low-dose group and Group III: high-dose group. p1: p value for comparing between group I and group II, p2: p value for comparing between group I and group III, p3: p value for comparing between group II and group III. *: Statistically significant at p ≤ 0.05. (SDI: sperm deformity index).
The mean blood glucose levels (mmol/L) are illustrated in Figure 3. Incense smoke inhalation caused a significant decrease in blood glucose levels in low and high-dose IS inhaling groups compared to the control unexposed group (P< 0.001). The mean blood glucose levels in the low and high doses and control unexposed groups were 7.9 ± 0.4, 7.8 ± 0.4, and 9.4 ± 0.6, respectively. On the other hand, the mean IS blood glucose levels in the high-dose group were insignificantly lower than that in the low-dose group (P= 0.945).

**Effect of IS on C-reactive protein (CRP) level**

The range and mean ± SD of CRP (mg/L) in the studied groups (Figure 4). Incense smoke caused a dose-dependent significant increase in CRP levels compared to the control group (P< 0.001). The mean CRP levels in low, high dose, and control groups were 25.6 ± 6, 35.3 ± 4.1, and 5.5 ± 2.7, respectively.

**Effect of IS on kidney function**

As presented in Figure 5A, there were statistically significant differences in the mean urea levels (mmol/L) between the studied groups. The mean urea levels in low and high-dose groups were dose-dependent and were significantly higher (P< 0.001) than that in the unexposed group (7.3 ± 0.6, 7.9 ± 0.3, and 5.5 ± 0.1, respectively). Similar to urea levels, the mean creatinine levels (µmol/L) in low and high-dose exposed groups were significantly higher (P< 0.001) compared to the unexposed group (50.4 ± 2.7, 53.05 ± 2.7, and 29.2 ± 2.7, respectively) (Figure 5B). However, there was an insignificant difference between creatinine levels in low-dose and high-dose groups (P= 0.275).

**Effect of IS on liver function**

Statistically significant differences in the mean ALT levels (U/L) were detected between the three studied groups (P< 0.001). The mean ALT levels in low and high-dose groups were significantly higher than that in the unexposed group (204.9 ± 32.41, 287.6 ± 25.37, and 107.2 ± 6.19, respectively) (Figure 6A). Moreover, the mean ALT level in the high-dose group was significantly higher than that in the low-dose group (P< 0.001). On the other hand, and similar to ALT levels, the means AST levels (U/L) in low and high dose groups were significantly (P< 0.001) higher than that in the control group (135.4 ± 2.46, 132.7 ± 5.64 and 114.3 ± 7.02, respectively). However, the low-dose and high-dose groups showed statistically insignificant differences (P= 0.621) (Figure 6B).

**DISCUSSION**

We showed that serum testosterone levels and sperm quality were negatively affected by prolonged IS inhalation in a dose-dependent manner.
Incense smoke inhalation affects spermatogenesis and sperm quality and impairs liver and kidney function in adult male rats

Moreover, prolonged inhalation of IS caused significant potential alterations in liver and kidney biochemical markers, suggesting potential toxicity. Regardless of the incense type, several studies have shown that emissions of air pollutants from burning incense contain many deleterious components including inorganic gases, particulate matter (PM), toxic organic compounds, and heavy metals (Jetter et al., 2002; Lee & Wang, 2004; Yang et al., 2012). Inorganic gas such as CO is known to cause toxicity due to its high affinity for hemoglobin, which causes a decrease in oxygen supply to tissues, resulting in hypoxia (Palmeri & Gupta, 2022).

Environmental exposure to hypoxia was reported to cause alterations in blood flow and oxygen supply along with an increased local temperature that may affect sperm quality (Reyes et al., 2012). Nitric oxide (NO), released from different types of incense was as high as 0.3 ppm, which is significantly higher than the acceptable levels of NO (<0.080 ppm) (Bootdee & Chantara, 2014). NO was found to induce mouse infertility in a time-dependent manner. Oxidative stress and apoptosis are involved in mediating NO-induced infertility (Wu et al., 2022). Nitrogen Dioxide (NO$_2$) and Sulfur dioxide (SO$_2$) induced the expression of oxidative-stress-related genes and were negatively associated with sperm quality in a population of men with infertility (Chen et al., 2020). Both NO$_2$ and SO$_2$ concentrations emitted from incense burning were found to surpass the standards stated by US National Ambient Air Quality Standards (NAAQS) (Jetter et al., 2002). Given the slow-burning process of incense, significant PM of different sizes is produced, and air pollution containing PM was previously reported to damage the human-sperm sex ratio (Radwan et al., 2018). The most common mechanism of action for all released toxins on spermatogenesis was systemic increases in oxidative stress, which in turn leads to aberrant inflammation and irreversible sperm DNA impairment, therefore affecting male fertility (Alahmar, 2019; Zhang et al., 2019; Huang et al., 2020; Kurkowska et al., 2020).

Maintenance of the structure and function of the male reproductive system are androgen dependent. Testosterone is necessary for the

Figure 5. Bar chart representing the mean value of urea levels (mmol/L) (A) and creatinine levels (µmol/L) (B) in all studied groups. The non-significant difference was ignored. *: Statistically significant at p ≤ 0.05, ***: Statistically significant at p ≤ 0.001.

Figure 6. Bar chart representing the mean value of ALT (A) AST (B) levels (U/l) in all studied groups. ***: Statistically significant at p ≤ 0.001.
spermatogenesis process and male fertility (Merghem et al., 2017; Walker, 2021). Therefore, any abnormalities and changes observed in this system may be linked to a testosterone deficiency (Walker, 2021). Our results indicated that testosterone levels in IS-exposed rats were significantly lower than that in the unexposed group. Exposure to air pollutants such as PM, CO and NO was reported to be negatively associated with the level of testosterone in a study performed on 327 men attending an infertility clinic (Radwan et al., 2016).

In andrology, it is believed that the main determinant of male fertility outcome is semen quality (Bonde et al., 1998). Computer-assisted semen analysis (CASA) showed that sperm progressive motility, velocity parameters, and vigor parameter (wobble %) significantly declined in IS-exposed rats compared to the unexposed group. The percentage of morphologically abnormal sperms significantly increased compared to the unexposed group. The sperm deformity index value in low-dose and high-dose groups was significantly higher than that in the unexposed group. Based on a study that demonstrated that fragile DNA showed a higher SDI value and vice versa (Aziz et al., 2011), our results indicate a more fragile DNA in IS-exposed groups. These may be due to the direct effect on testicular tissue which results in reproductive dysfunction such as reduced sperm progressive motility, velocity, and vigor parameters as well as sperm morphological abnormalities. A previous report demonstrated that *Carlina gummifera* IS inhalation caused oxidative stress in the testis, which may be the indirect cause of the observed changes in spermatogenesis (Dorsaf et al., 2022).

Blood glucose is an indicator of the healthy state of the organism, our results indicated that blood glucose level was significantly decreased in IS-exposed rats compared to the unexposed group, suggesting liver damage as recently indicated by Bai et al. study which demonstrated that the levels of fasting blood glucose and 2 h postprandial glucose changed in liver cirrhosis. Glycogen concentrations and the activity of glucose-6-phosphatase in the liver were decreased (Bai et al., 2021). Epidemiological studies suggest that exposure to carbon monoxide, nitrogen dioxide and PM from outdoor or traffic-related air pollution is related to a greater risk of chronic kidney and liver disease (Kim et al., 2019). The kidneys and liver’s normal function is not only important for physiological processes such as detoxification, metabolism, protein synthesis, blood sugar balance, waste and excess fluid elimination and regulation of blood pressure but most importantly to the scope of this manuscript, kidney or liver disease were reported to affect sperm production and quality (de Kretser, 1997). Serum hepatic enzyme activity was negatively related to sperm concentration and total sperm count (Elghar, 2013). It was demonstrated that wood smoke PM can cause liver damage through the generation of reactive oxygen species, lipid peroxidation, genotoxicity and endoplasmic reticulum stress (Danielsen et al., 2010; Laing et al., 2010).

Regarding the effect of IS inhalation on liver function, results indicated that blood ALT and AST levels were significantly increased in IS-exposed rats compared to the unexposed group. This suggests that IS inhalation may cause liver damage where the liver is susceptible to damage from direct exposure to toxic products due to excessive detoxification of toxins and xenobiotics (Abd-Elhady & Abou-Elghar, 2013). It was demonstrated that wood smoke PM can cause liver damage through the generation of reactive oxygen species, lipid peroxidation, genotoxicity and endoplasmic reticulum stress (Danielsen et al., 2010; Laing et al., 2010).

Urea and creatinine are excreted primarily by the kidney so their blood levels serve as indicators of renal function. Blood urea and creatinine levels were significantly increased in IS-exposed rats compared to the unexposed group in a dose-dependent manner. This finding suggests that IS inhalation may be associated with a disturbance of renal function. The mechanism underlying the link between IS inhalation and renal disease might be explained by Hussain et al study, which indicated that IS-exposed rats had deterioration in normal physiological functions with a substantial increase in serum creatinine, uric acid, and blood urea nitrogen, and significant ultrastructural changes in kidney tissues. This was
Incense smoke inhalation affects spermatogenesis and sperm quality and impairs liver and kidney function in adult male rats. This is accompanied by a corresponding increase in inflammatory markers such as tumor necrosis factor-alpha and interleukin-4 levels, an increase in oxidative stress indicated by a significant increase in tissue malondialdehyde and tissue gene expression of both CYP1A1 and CYP1A2, and a decline in tissue reduced glutathione and catalase activity (Hussain et al., 2016). Additionally, our study showed that the mean of serum CRP levels in IS-exposed rats was significantly higher than that in the unexposed group in a dose-dependent manner. Moreover, it was reported that CRP is not only an inflammation biomarker but also an activated mediator of acute kidney injury. It exerts undesirable effects on the injury processes and affects repairing through multiple mechanisms (Tang et al., 2018). Collectively, we suggest that the adverse modulating effects of IS inhalation on renal functions in rats may be mediated through augmented inflammation and oxidative stress.

Collectively, our findings indicated that IS inhalation may cause kidney and liver damage. Moreover, it can affect sperm quality including progressive motility, velocity, and vigor parameters, and cause abnormal morphological changes. However, further studies are required to explain the molecular mechanism of IS-induced cytotoxic effects.

**CONCLUSION**

This study shows that prolonged IS inhalation may affect male fertility potential where it significantly reduced sperm progressive motility, velocity, and vigor parameters and also significantly affect sperm morphology. Moreover, it significantly affects kidney and liver function as well as blood glucose levels.

**ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

All animal procedures and experimental protocols were approved by the Research Ethics Committee of the medical research institute, Alexandria University (AU0122282233). All animal experiments were performed according to the ARRIVE guidelines and the National Research Council’s guide for the care and use of laboratory animals.

**AVAILABILITY OF DATA AND MATERIALS**

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**COMPETING INTERESTS**

The authors declare that they have no competing interests.

**FUNDING**

The study is self-funded.

**AUTHORS’ CONTRIBUTIONS**

T.S. & N.F. designed the research proposal idea and performed the practical part of the research. N.F. and S.E. analyzed and interpreted the results of this study. T.S. and S.E. wrote the main manuscript text. All authors read and approved the final manuscript.

**REFERENCES**


Lima, G. D. A., Sertorio, M. N., Souza, A. C. F., Menezes, T. P., Mouro, V. G. S., Gonçalves, N. M.,
Incense smoke inhalation affects spermatogenesis and sperm quality and impairs liver and kidney function in adult male rats


Environmental Science and Pollution Research, 23(23), 23339-23348.


