



Arsenic Accumulation in the Lung, Liver, And Kidney of Rats Chronically Exposed to Cigarette Smoke

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ABSTRACT:

Introduction: Cigarette smoking is a significant source of arsenic exposure for men through inhalation. The exposed rats had higher carboxyhemoglobin levels in their blood and received a daily dose of total particulate matter (TPM).

Objectives: To study the Arsenic distribution and accumulation in different system due to smoke exposure

Methods: Male albino rats were exposed daily for 40-45 weeks to mainstream smoke from Marlboro Light cigarettes in a special nose-only exposure system

Results: The results indicated effective inhalation of smoke by the rats. Tissue samples were analysed for arsenic using atomic absorption spectrometry after acid digestion. The exposed rats showed 64- and 2-3-fold higher levels of arsenic in their lungs and kidneys than control rats. In contrast, the levels of arsenic in the liver did not show a significant increase

Conclusions: The data suggests that chronic inhalation of low-dose cigarette smoke leads to the highest accumulation of arsenic in the lungs, followed by the kidneys, with minimal impact on the levels of arsenic in the liver.

1. Introduction

According to [1] arsenic is acknowledged as a major environmental contaminant that significantly affects human and other species' health. Both tobacco use and occupational exposure contributed to the elevated levels of inorganic arsenic exposure [2]. It has been noted that second-hand and tobacco smoke, which the IARC has identified as carcinogenic substances for lung tissues [3], may be more dangerous for individuals who are concurrently co-exposed to arsenic (ingested or breathed). A strong carcinogen, inorganic arsenic causes

cancer in the liver, kidneys, skin, lungs, and bladder [4]. A large amount of this metal is transported to smoke during cigarette burning, which the smoker inhales. Although it is well known that smokers have higher tissue arsenic contents than non-smokers do, rigorous experimental research has not been done to ascertain how much-inhaled smoke contributes to arsenic loads in various organs.

Hepatomegaly from cirrhosis and ascites is a typical clinical manifestation of arsenic poisoning, which primarily affects the liver [5]. Serum albumin (ALB),



alanine aminotransferase (ALT), and total bilirubin (TBIL) are early biological markers showing liver damage in the population affected by arsenic poisoning, according to earlier research [6,7]. Additionally, arsenic-induced liver damage is largely caused by oxidative stress brought on by decreased activity of antioxidant enzymes (such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and sulfhydryl (-SH)) [8,9] This leads to an increase in the production of malondialdehyde (MDA), a by-product of lipid peroxidation.

2. Objectives

The goal of the current investigation was to ascertain the relative tissue load of arsenic in experimental rats exposed to tobacco smoke for an extended period daily, nose-only, under carefully monitored conditions.

3. Methods

Two weeks were spent observing male Wistar rats in isolation chambers. Healthy animals were chosen at random (6 animals per group) for the studies and kept in bio-clean rooms with HEPA filters that were environmentally regulated. The animals were kept in hanging cages made of stainless steel wire and exposed to a 12-hour daily light cycle. The air exchange rate in animal rooms was kept at 40 times per hour to reduce the number of extraneous particulates that the animals were exposed to. Water and Purina Rodent Chow were available to all animals at all times, except for exposure. Animals were separated into the three categories listed below.

- (i) Room controls (RC), are handled once a week for regular cage cleaning;
- (ii) sham (SH)-treated group, receives daily treatment similar to that of the smoke-exposed group but without smoke;
- (iii) Smoke-exposed (SM) group, is exposed to fresh mainstream smoke from high-tar/high-nicotine (Marlboro) cigarettes only through the nose. Each cigarette in this batch included $1.23 \pm 0.1 \mu\text{g}$. Every cigarette produced an average of $0.26 \pm 0.03 \mu\text{g}$ of mainstream particles, according to an examination of the mainstream smoke from these cigarettes.

For 25–30 weeks in a row, the rats were exposed once or twice a day, seven days a week. Every time, the exposure

period lasted for 10 minutes. A detailed description of the exposure system, exposure circumstances, and smoke inhalation marker measurement has already been provided elsewhere [10].

The animals were sacrificed by aortic exsanguination, after which they were put to sleep with an intraperitoneal dose of pentobarbital. After removal, the kidneys, liver, and lungs were washed in deionized distilled water and refrigerated at -80°C until the assay. The tissues were vacuum-dried in an oven at 75°C until they reached a constant weight, and their weights were noted to determine the amounts of arsenic. Following that, the tissues were reflexed at 100°C for a whole night in a mixture of nitric acid and perchloric acid (9:1). After drying off the digests, the leftovers were reconstituted in 0.1 N hydrochloric acid. Flame atomic absorption spectroscopy (Instrumentations Laboratory, S-11 spectrophotometer) [11] was used to measure the amount of arsenic in the tissue digests. The data were statistically analyzed using variance analysis and the multiple comparison test developed by Newman-Keul.

4. Results

The animals treated with sham or smoke had considerably lower body weights than the room controls, indicating possible treatment-related overall stress effects (Table 1). The rats that were exposed to smoke had a daily total particulate matter (TPM) intake and carboxyhemoglobin (COHb) readings that showed that they were exposed to more than double the amount of smoke particulates (based on body weight) as rats (Table 1). The smoke-exposed groups of both species had significantly higher pulmonary aryl hydrocarbon hydroxylase (AHH) activity than the room or sham control groups (Table 2). These findings proved that during the exposures, the animals had ingested cigarette smoke. After more than 6 months of daily exposure to reference cigarette smoke, the amounts of metals were examined in three different rat tissues. Fig. 1 shows the weight of rats' liver, lungs, and kidney. These organ weights significantly changed when compared with those of their respective control rats. Fig. 2 displays the amounts of arsenic in rats' liver, lungs, and kidneys. These organs' arsenic contents from the room control and sham-treated animals did not differ significantly. Among the three organs.



Rat kidneys had the highest basal amounts of arsenic that were measured. Animals exposed to smoke had far higher levels of arsenic in their kidneys and lungs. In comparison to control groups, there was an average 5-fold rise in arsenic levels in the lungs and a 2.5-fold increase in the kidneys across both species. In the two animal species this study looked at, there was no correlation found between smoking exposure and liver arsenic levels.

5. Discussion

The primary goal of the current investigation was to ascertain if long-term inhalation exposure to mainstream cigarette smoke under precisely defined smoke generation and exposure circumstances results in the build-up of arsenic in the lungs, liver, and kidneys of male rats. The findings indicate that exposed animals of species had a notable build-up of arsenic in their kidneys and lungs, but not in their livers. Currently, it is unclear how arsenic can accumulate in the kidney without also increasing in the liver. The animals were exposed to an average of 3.2 and 7.24 mg kg⁻¹ body wt. of smoke particulate matter per exposure for rats. These figures show a modest smoke dose in comparison to human smokers, as an adult human smoker who smokes one pack per day is estimated to receive 6.8 mg of smoke particles kg⁻¹ body weight [12] When compared to their corresponding controls, exposed animals' blood COHb and lung AHH levels significantly increased, indicating that the animals had breathed both the gaseous and particle phase constituents of cigarette smoke.

Increased amounts of arsenic have been found in smokers' bodily fluids and tissues in several human investigations [13,14]. Smokers' lung, kidney, and liver tissues had an elevated arsenic concentration of 1.7, 2.0, and 1.6 times that of non-smokers' tissues, respectively [15]. In the current study, rats exposed to mainstream cigarette smoke via their noses alone for a year showed approximately 5- and 2.5-fold increases in arsenic levels in the kidney and lung, respectively. According to [16, 17] rats' poorer lung clearance of smoke particles may be the cause of the significant arsenic deposition in the lungs of exposed animals. Cigarette smoke particulates are known to have the ability to bind metals, and each cigarette is known to bind about 800 µg of arsenic. [18] The amount of arsenic in the lungs will therefore rise if these particles are not effectively eliminated. It is

noteworthy that the current study's arsenic transfer from cigarette tobacco to mainstream smoke was about 12%, falling within the range (4–25%) documented in the literature. Both [12,19] It's also crucial to note that the study's reference cigarettes' arsenic level (2.13 pg per cigarette) was marginally higher than the values for commercial cigarettes (1-2 µg per cigarette Samuel Samuel e) [20] which could have led to elevated arsenic levels in the exposed animals' lungs.

The findings of this study show that rats inhale low-dose mainstream cigarette smoke on a daily basis have far higher levels of arsenic in their kidneys and lungs than in their liver.

Conclusion:

The current investigation showed that rats' lung, liver, and kidney tissues significantly accumulate arsenic as a result of long-term exposure to cigarette smoke. This accumulation was found to be dose-dependent, with higher levels of cigarette smoke exposure correlating with greater arsenic deposition in these organs. The largest concentrations of arsenic build-up were seen in the lungs, consistent with exposure from direct breathing. This implies a direct relationship between lung damage from cigarette smoke and the build-up of arsenic in lung tissue. Significant arsenic accumulation was also seen in the kidney and liver, suggesting systemic absorption followed by excretory and detoxifying mechanisms. The results highlight the dangers to one's health posed by long-term exposure to tobacco smoke, especially the build-up of harmful substances like arsenic in critical organs. The report emphasizes how crucial it is to implement public health initiatives to lower cigarette smoking and lessen its toxic effects.

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Conflict of Interest – The authors declare that they have no conflict of interest.

Author's contribution- Sandeep Tripathi - Designed the model and the computational framework and analyzed the data. Devesh Kumar Joshi -Carried out the experiment and wrote the manuscript. Sonalika Singh-



contributed to the interpretation of the results. **Divya Darak**- helped in carrying out the experiment

Ethics Statement – I have uploaded animal Ethical consent for this study in a supporting file.

Informed consent – I have uploaded animal Ethical consent for this study in a supporting file.

Availability of data and materials: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Table 1. Body weights.

intake		Blood COHb		Daily TPM
Body wt. (g)		(%)		(mg/kg body wt.)
RC	SH	SM	SH	SM
498±52	415±46	360±38	≤1	7.2±0.6
				3.2±0.8

·RC = room control; SH = sham-treated; SM= smoke-exposed

Table 2. Pulmonary aryl hydrocarbon hydroxylase (AHH) activity.

Pulmonary AHH activity
(pmol ³H-benzopyrene metabolized min⁻¹
Mg⁻¹ protein)

RC	SH	SM
1.05±0.06	0.98±0.03	2.20±0.18

·RC = room control; SH = sham-treated; SM= smoke-exposed

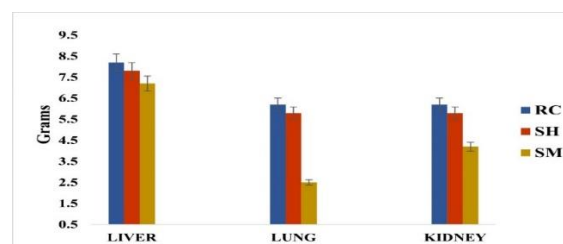


Figure 1

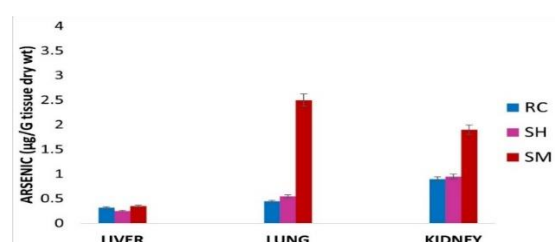


Figure 2

Figure 1. Organ weight of liver, lung, and kidney.

Figure 2. Arsenic content of liver, lung, and kidney of rats