APOPTOTIC AND STRUCTURAL CHANGES IN LUNGS TISSUES OF TOLUENE INDUCED RESPIRATORY INJURY IN WISTAR RATS

Abstract.

To investigate the impact of toluene exposure on the functional-morphology of the pulmonary tissues, 35 Wistar rats were acquired. The study was divided into three (3) groups: A, B, and C, with three (3) phases: acute (7 days), subchronic (14 days), and chronic (21 days). Group A was used as a control group, and they were given rat food and tap water ad libitum. Group B was given 200ml of toluene and observed for seven days, fourteen days, and twenty-one days. Group C was given 400ml of toluene and was kept under observation for seven days, fourteen days, and twenty-one days. The animals were euthanized after three weeks of exposure, with each exposure lasting 60 minutes each day. Finally, the lungs of each group were removed for histological examination. The study animals' mean body weights reduced significantly ($P<0.05$) when compared to the control group, according to the findings. In contrast to group A, histopathological evaluation of all treatment groups indicated pulmonary tissue inflammation and necrosis in groups B and C. Finally, the findings of this investigation revealed that toluene exposure can affect Wistar rats' lung functioning and morphology.

Keywords: Toluene, Apoptosis, Lungs tissues, Respiratory injury

Introduction

When tissues are exposed to various substances or foreign materials, they undergo alterations that might be beneficial or harmful. Toluene, also known as toluol, methylbenzene, and phenylmethane, is the most prevalent aromatic hydrocarbon in the environment. Toluene is found in a variety of home products, including paints, paint thinners, silicon sealant, various chemical reactants, printing ink, adhesives, lacquers, leather tanners, and disinfectants.

Inhalation, skin contact, and consuming water and food are the main routes of exposure to the body. Toluene is easily absorbed by the respiratory and gastrointestinal tracts, as well as the skin to some extent. Although, pulmonary absorption is quicker and accounts for 40-60% of toluene ingested [(1)]. Dermal absorption is sluggish, ranging between 14 and 23 mg/hr at the forearm [(2).]

Toluene is found in small amounts in crude oil and as a by-product of the catalytic reformer or ethylene cracker used to make gasoline. It's also a by-product of coal-based coke manufacture before being separated and purified. Toluene is also used as an octane booster in gasoline fuels for internal combustion engines and aviation fuel (jet A1).

Toluene became so popular in combustion engines that it was discovered in 2003 in Australia that it was illegally mixed with petrol in commercial outlets as standard vehicular fuel

because it incurred no government tax, unlike petrol, which was taxed at 40%, allowing fuel suppliers to make a larger profit margin.

Toluene can also be used in the laboratory to split open red blood cells and extract haemoglobin for biochemistry investigations [(3)].

Because toluene can pass through the placenta, it can cause microcephaly, preterm delivery, growth retardation, and head and facial abnormalities in the fetus if it is exposed during pregnancy. Inhalation can cause foetal toxicity and impaired bone development [(4).]

Because toluene is lipophylic, it can pass across the blood-brain barrier and have neurological consequences.

Interstitial widening, a drop in neutrophils, degenerated lymphocytes, and other leukocytes were observed as the quantity of toluene increased in a study by Adeli et al [(5).] The increase in leucocytes was ascribed to the discharge of immature cells into the bloodstream. The thickness of the bronchial wall, as well as congestion of the alveolar parenchyma, increased with increased exposure.

However, it has been revealed that the principal toxic effect of toluene on the alveoli is linked to glutathione levels, with the resultant effect of DNA damage and cell death. Reduced gas exchange between the septa, internal alveolar space, and surrounding capillaries has a negative effect on type 1 pnuemocytes [(5).]

A degradation of type 2 cells was found in another study by Ishiola et al., [(6)] which increased with larger concentrations of paint fumes exposure. The deterioration of clara cells in the lungs of automotive paint sprayers was observed by Abuelfadl et al in 2010. There was also no evidence of airway epithelium injury or inflammatory cell infiltration. This could indicate that the earliest lung response to paint fume exposure is a reaction centered on the pulmonary microvasculature that is unknown.

Duninho et al., (2002), on the other hand, found alveolar and interstitial flooding in the lungs of mice exposed to phosgene, a volatile organic chemical, along with inflammatory cell infiltration and terminal airway epithelial degradation.

In the brain tissues of rabbits exposed to toluene, Mehmet Demur et al. (2017) discovered focal vacuolar degeneration, gliosis, perivascular demyelination, and numerous pyknotic cells and necrosis. The overall tissue structure was also severely damaged, with dispersed cell boundaries and aberrant malformation of the nucleus structure of oligodendrocyte cells.

Toluene enhances apoptotic activity in the tissue, according to Tas U. et al., [7] who came to this conclusion after immunochemistry results demonstrated an increase in Bax and C3 activity in such exposed tissues.

Caspase-3 activity increased in tissues exposed to toluene, according to El-Nabi Kamel and Shehata [8], (an enzyme activated in apoptotic process). Chronic exposure to toluene, according to Tas et al. [7], had a considerable increase in TUNEL positive cells in their lungs, brains, and testicles.

Although Calderon et al., [11] suggested that toluene increases membrane fluidity by altering the lipid structure of the cell membrane, thereby affecting the Na/K-ATPase activity, Karabulut et al., [9] and Lee et al., [10] found that toluene causes tubular damage by increasing oxygen radicals. Meydan et al. [12] and Bowen et al. [13] investigated the effects of toluene on the GABAergic, glutamatergic, serotonergic, and dopaminergic pathways in high doses.

Despite publications on the impact of toluene on numerous organ systems, there are few studies on the influence of toluene exposure on the apoptotic and histological alterations of pulmonary tissues in Wistar rats. The current examination is justified in light of this.

Materials and Methods

Ethical approval

Our institution's ethics committee gave its approval to the study. The group created international standards, norms, and recommendations for the use of animals in research [14].

Preparation of toluene

SUNYBROS chemical company (nig.), 195 Faulks Rd. Aba, Abia State, provided the toluene for this investigation. It was checked for originality by the chemical department of Abia State University.

Picture of Toluene

After the toxicity level of toluene was validated using the LD50 test, the amount of toluene was measured in millilitres using the measuring cylinder according to each group ratio.

Experimental Design

A total of 35 rats weighing 150-200g were obtained from Abia state animal farms, Uturu, Nigeria. The rats were kept in wire gauze cages and given two weeks to acclimate before being given the medication. The rats were fed rat chow and had access to water during the experiment. The study was divided into three (3) groups: A, B, and C, with three (3) phases: acute (7 days), subchronic (14 days), and chronic (21 days). One compartment held five (5) rats in Group A, which acted as the control group. The test groups, B and C, had three compartments, each holding five (5) rats.

Animal Groupings

Group A (Control group) were given rat meal and tap water ad libitum only.

Group B was given 200ml of toluene for an hour and then monitored for seven days, fourteen days, and twenty-one days.

Group C was given 400ml of toluene for an hour and then observed for seven days, fourteen days, and twenty-one days.

The animals were sacrificed after the trial lasted three weeks.

Collection and Preparation of Lung Tissues for Analysis

The rats were sacrificed utilizing the cervical dislocation every week as mentioned in the experimental period. The rats were placed on a dissecting board and dissected quickly with dissecting kits, with the study organs removed. Each week, four rats from each group were utilized for lungs histology, which were fixed in 10% formal saline.

Statistical Analysis of Results

M ± SEM of triplicate measurements were used to calculate the results. All of the data was analyzed using the Statistical Package for Social Sciences (SPSS) version 23. (IBM Corp, Armonk, New York). At the $P<0.05$ significance level, hypotheses were evaluated by looking for significant differences.

RESULTS

Table 1: Values of Body weight of toluene in study animals

Groups	Day 0	Day 7	Day 14	Day 21
A (control)	160.00± 0.00	170.40±1.44	201.60±2.84	225.40±9.70
B (Toluene 200ml)	160.00± 0.00	144.00±4.56[a]	120.80±3.18[a]	132.40±7.11[a]
C (Toluene 400ml)	160.00± 0.00	152.80±6.37[a]	115.80±3.83[a]	113.80±5.42[a]

Key: values are presented as mean ± sem. N=5. **a =** mean values are statistically significant compared to control.

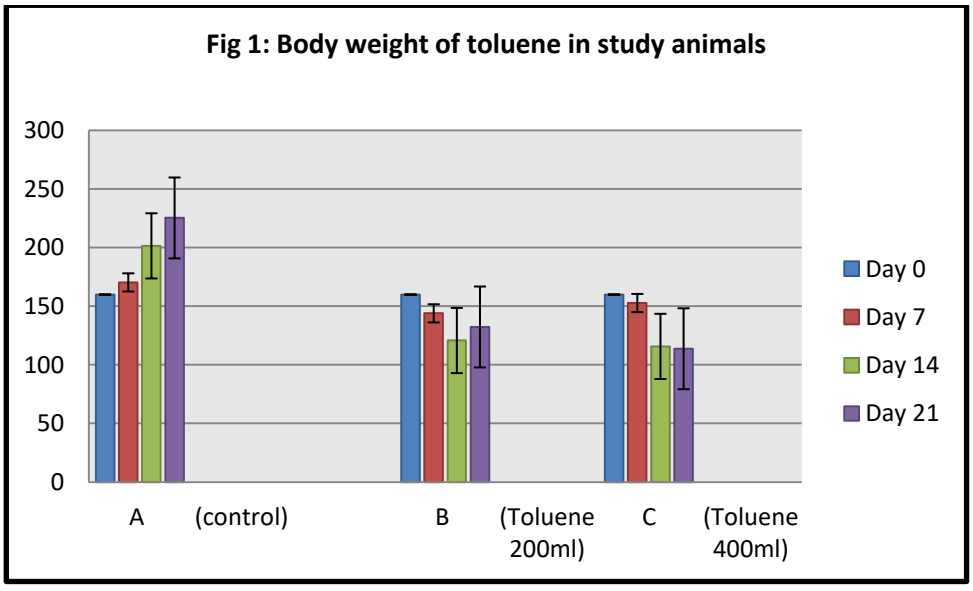
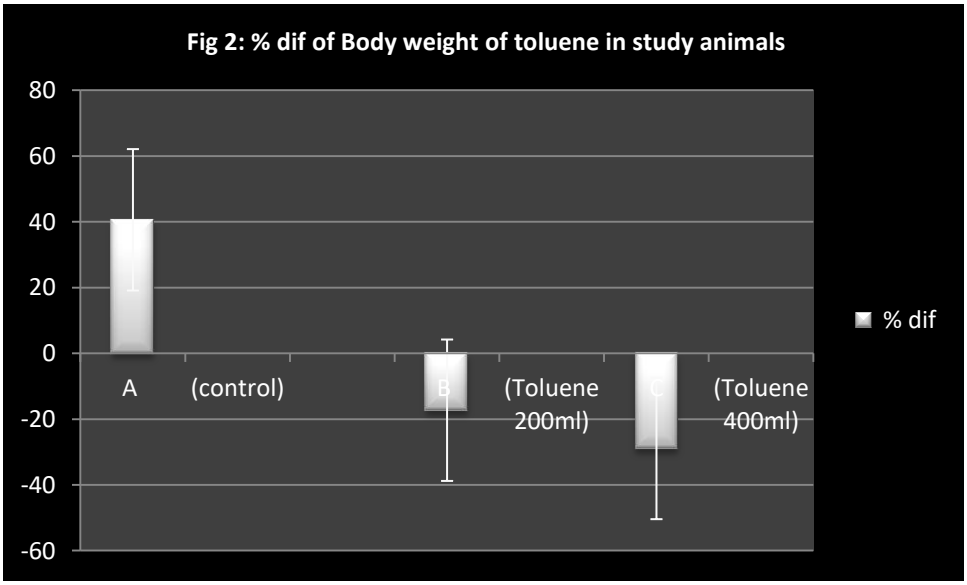


Table 2: Percentage difference of Body weight of toluene in study animals

Groups	% difference
A (control)	40.65
B (Toluene 200ml)	-17.25[a]
C (Toluene 400ml)	-28.875[a]

Key: values are presented as mean ± sem. N=5. **a =** mean values are statistically significant compared to control.



When compared to the control, the body weights of animals in the test groups (B and C) showed a statistically significant decrease. In addition, our results show a negative percentage difference in body weights, indicating that group C suffered more damage than group B.

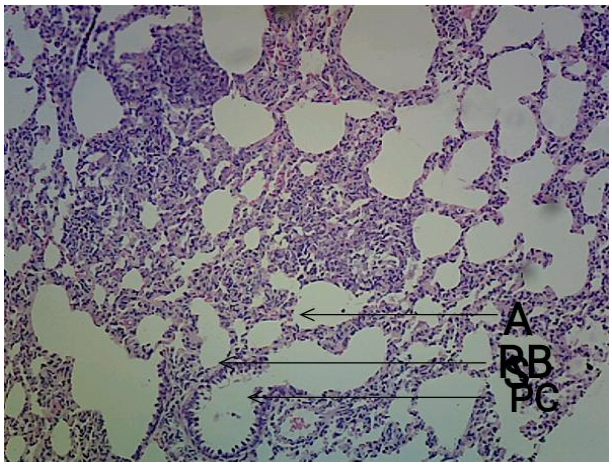


Plate A (Control) H&E

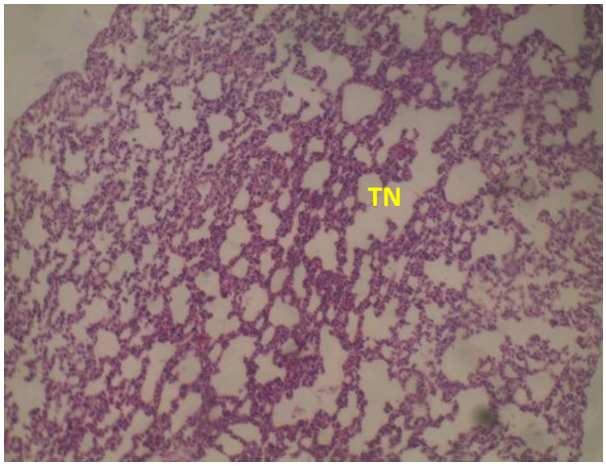


Plate B (Day 7) H&E x 125 Mag

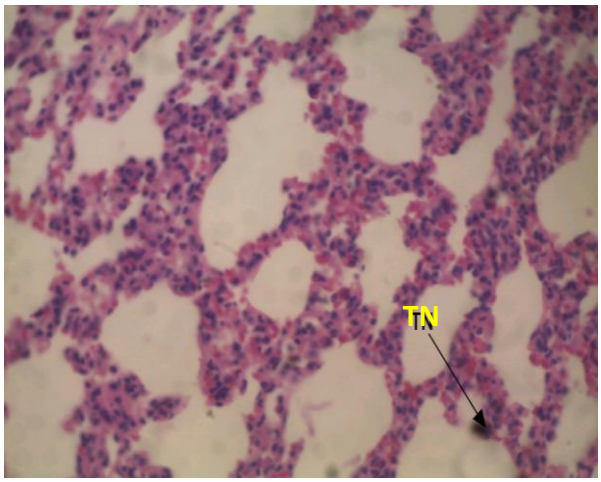


Plate B (Day 7) H&E x 600 Mag

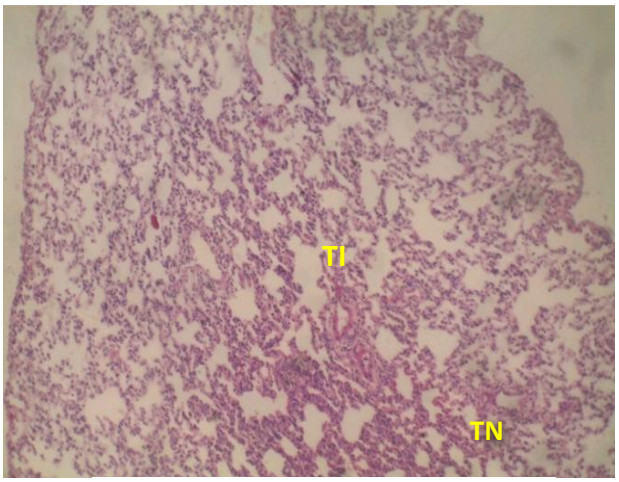


Plate B (Day 14) H&E x 125 Mag

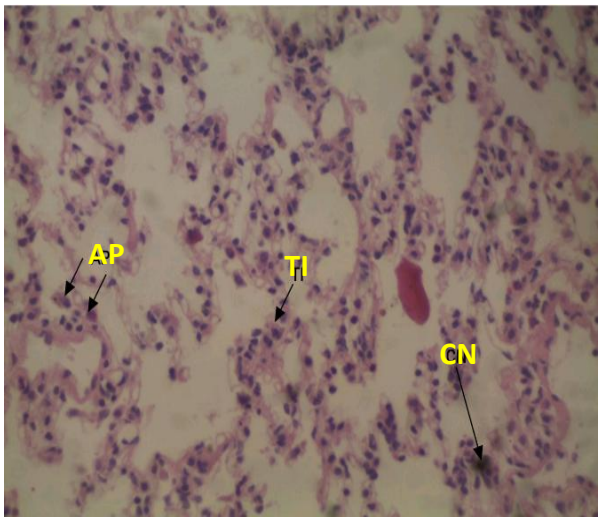


Plate B (Day 14) H&E x 600 Mag

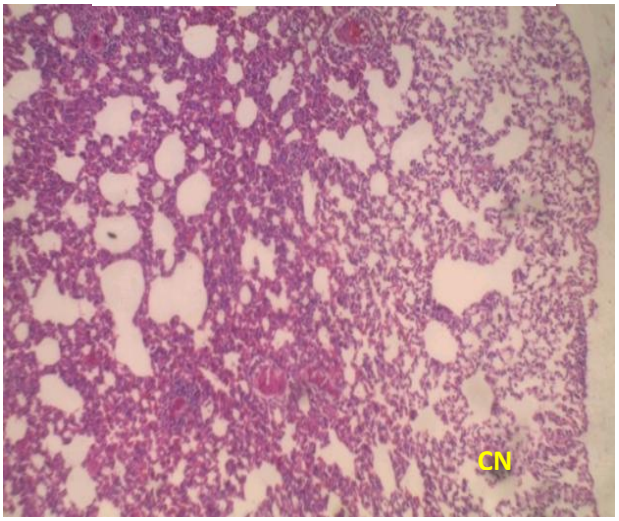


Plate B (Day 21) H&E x 125 Mag

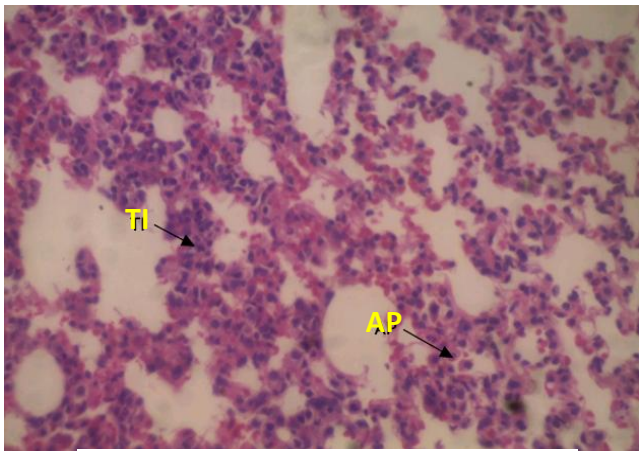


Plate B (Day 21) H&E x 600 Mag

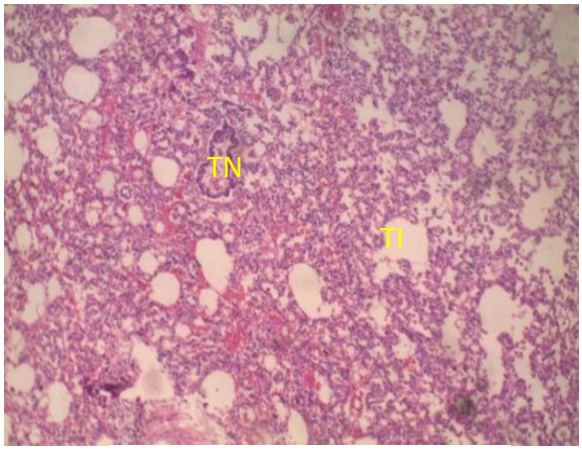


Plate C (Day 7) H&E x 125 Mag

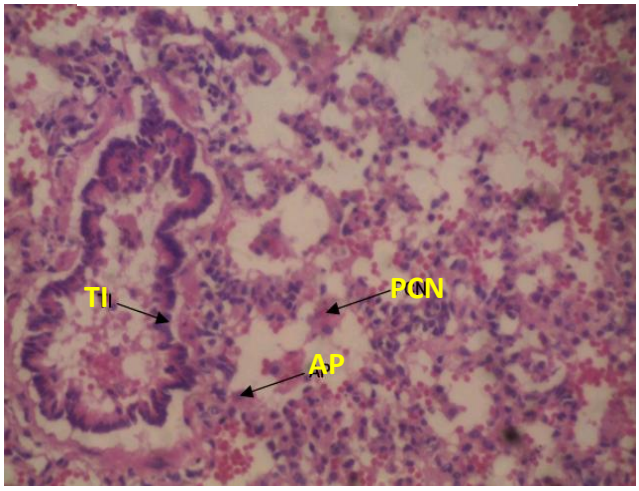


Plate C (Day 7) H&E x 600 Mag

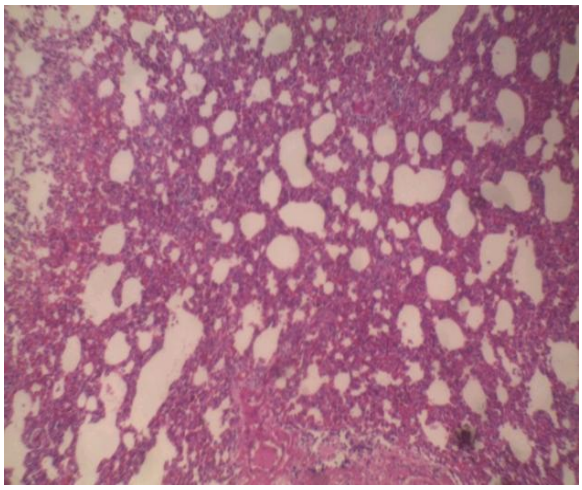


Plate C (Day 14) H&E x 125 Mag

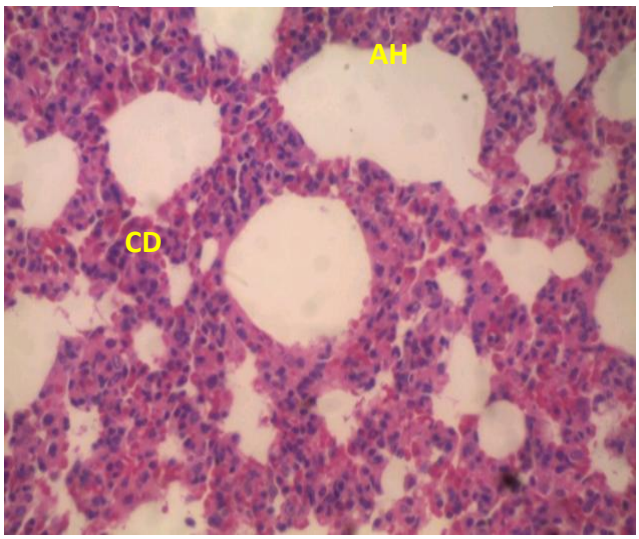


Plate C (Day 14) H&E x 600 Mag

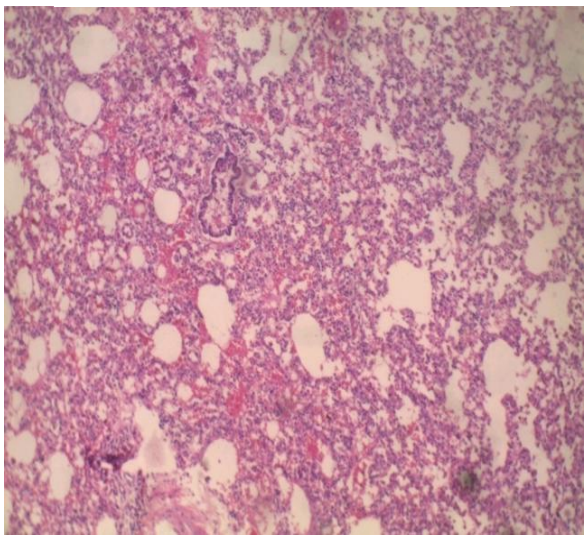


Plate C (Day 21) H&E x 125 Mag

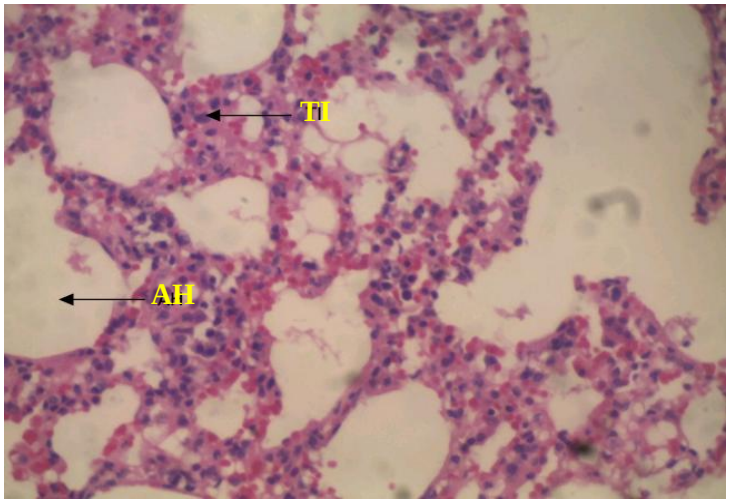


Plate C (Day 21) H&E x 600 Mag

Plate A depicts normal lung tissue with a pronounced respiratory bronchiole (RB) surrounded by pneumocytes (PC) in rat lungs from the control group. The alveolar sac is also present (AS). Plate B: depicts a photomicrograph of rat lungs taken on Day 7 (week 1) with moderate tissue inflammation and early evidence of tissue necrosis (x125), as well as the initiation of tissue inflammatory reaction and tissue necrosis (TN) (x600). On Day 14 (week 2), there was a greater degree of tissue inflammatory reactivity. Evidence of tissue shrinkage is used to diagnose tissue necrosis (x125). At high magnification (x600), the plate shows significant lung tissue distortion with advanced stage tissue inflammatory reaction (T.I) and cellular necrosis (CN). This suggests apoptosis (AP). On Day 21, a lower magnification photomicrograph of the rat's lung reveals that the tissues are still in an advanced stage of inflammatory reaction. The photomicrograph of Day 21 at higher magnification (x600) shows clearer signs of advanced tissue inflammatory reaction, apoptosis, and necrosis. At x125, a photomicrograph of a rat's lung in plate C (Day 7) shows areas of tissue inflammation (TI) and tissue necrosis (TN). Aside from that, the tissue appears to be normal. Plate C (Day 7) shows areas of tissue inflammation (TI), pockets of cellular necrosis (PCN), and an early state of apoptosis (AP) at a greater magnification (x600). The photomicrograph in plate C (Day 14) at low magnification (x125) demonstrates more severe tissue inflammation (arrow heads). The overall tissue architecture appears deformed and fuzzy. The photomicrograph of plate C (Day 14) at higher magnification (x600) indicates cellular confusion and more acute inflammation. The photomicrograph of a rat's lung at x125 in Plate C (Day 21) demonstrates severe tissue inflammation and alveolar congestion, as well as a severe stage of apoptotic reaction. The photomicrograph of Plate C (Day 21) at greater magnification (x600) demonstrates tissue inflammation and alveolar hypertrophy.

Discussion

The Wistar rats were separated into three groups in this investigation (A, B and C). The results revealed that group A (the control group) gained more weight over the course of the trial. In contrast, group B rats exposed to 200ml of toluene and group C rats exposed to 400ml of the same molecule showed a statistically significant percentage weight loss compared to control rats. This depicts the association between weight and toluene exposure. Dick et al., [15] discovered a link between prolonged inhalation of toluene and weight loss, since it has a long-term effect on metabolic activities, potentially increasing the risk of energy balance and glycemic control issues. This is in line with the current study's findings.

The histology of the lungs revealed unequivocal evidence of tissue damage at varied toluene concentrations. A photomicrograph of typical lung tissue architecture was shown in the control group (A), which had prominent respiratory bronchioles (RB), type 2 pneumocytes (PC), and an alveolar sac. The other two groups (B and C) treated to diverse concentrations of toluene for varying lengths of time showed a lot of distortion in the architectural display of the tissues. The group B rats treated to 200ml toluene for 1hr in the acute (7 days), subchronic (14 days), and chronic phase (21 days) showed early initiation of tissue inflammation and necrosis, as demonstrated by tissue shrinkage, indicating apoptosis at higher magnification. The distortion grew more obvious as the period of exposure increased in group C, which had a significantly greater concentration. Apoptosis (programmed cell death) became more prominent in this group (C). Rats exposed to paint fumes developed alveolar fibrosis, which advanced as the week of exposure rose, according to a study by Ishiola et al., [6]. Finally, two critical parameters determined the severity of tissue damage, such as tissue inflammatory alterations, necrosis, and apoptosis: the concentration of toluene and the period of exposure. Toluene, while useful and found in almost everything from car paints to resins, furniture varnish, shoe gums, and industrial adhesives, should be handled with caution because the immediate catastrophic effect of this harmful compound could pose a threat to human health, even if the threat is minor or insignificant depending on exposure time. When workers in the shoe industry, automotive paint sprayers, or even furniture makers are exposed to it on a regular basis, it raises concerns. When faced with the obstacles of exposure owing to occupational demands, safety practices, such as wearing face gear, should be followed.

Conclusion

Our research has shown that inhaling this unnoticed substance – toluene – has an effect on the pulmonary tissues, indicating that there is a pressing need to educate the public about this effect,

particularly first-hand users such as painters, carpenters, and shoe repairers, as well as provide additional advice on other protective measures of usage and lighter effect chemicals that may be helpful.

Competing Interests

There are no competing interests stated by the authors.

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