

Comparative Effects of Inhaled Gasoline Fumes on The Femoral Bone of Wistar Rats of Both Sexes

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Abstract—

Background: Gasoline fumes contain volatile hydrocarbons capable of producing toxic effects following prolonged exposure. However, information on their effects on the femoral bone histoarchitecture of male and female Wistar rats remains limited. This study investigated the comparative effects of inhaled gasoline fumes on the femoral bone of Wistar rats of both sexes.

Materials and Methods: Fifty-two (52) adult albino Wistar rats comprising twenty-six males and twenty-six females (135 ± 4 g) were used for the study. The animals were assigned into test and control groups according to sex. The test groups were further divided into rats exposed to gasoline fumes for 6 hours daily (Group A) and 9 hours daily (Group B), while Group C served as the control. The rats were acclimatized for five days and maintained under standard laboratory conditions with free access to food and water. Gasoline fume exposure was carried out using the method described by Uboh et al. (2007). At the end of the exposure period, the femoral bones were harvested, fixed in 10% formalin, processed histologically, decalcified, sectioned at 5 μ m, and stained using Hematoxylin and Eosin. Histological examination was performed using a light microscope with photomicrograph facilities.

Results: Histological examination showed no observable abnormalities in the femoral bone histoarchitecture of rats exposed to gasoline fumes for either 6 or 9 hours daily. The osteocytes retained normal morphology, and the trabecular architecture remained intact in both exposure groups. Likewise, the control animals exhibited normal femoral bone histology. Comparison between male and female rats revealed no apparent sex-related differences in the histological response to gasoline fume inhalation. The study recommended that further studies should evaluate the effects of longer exposure periods and different concentrations of gasoline fumes on the femoral bone. Also future research should employ additional techniques, such as bone mineral density and biochemical analyses, to detect subtle bone changes

motorcycles, power generators, and industrial equipment worldwide. It is a volatile and highly flammable mixture containing more than 500 hydrocarbons, including toxic constituents such as benzene, toluene, ethylbenzene, and xylene (BTEX), which readily evaporate into the atmosphere during storage, transportation, and combustion (Caprino et al., 1998). Consequently, gasoline fumes have become a common environmental and occupational pollutant, particularly in developing countries where dependence on fuel-powered generators and petroleum-related occupations is high.

Human exposure to gasoline fumes occurs mainly through inhalation during vehicle refueling, fuel transportation, automobile repair, oil refining, and prolonged exposure to traffic emissions. Individuals such as fuel station attendants, petroleum refinery workers, mechanics, tanker drivers, and residents of heavily industrialized or traffic-congested areas are especially vulnerable because of repeated and prolonged exposure (Rothman et al., 2016). Once inhaled, gasoline constituents enter the bloodstream through the lungs and are distributed to different organs, where they undergo metabolic transformation into reactive intermediates capable of inducing tissue injury and oxidative stress.

Several studies have demonstrated that gasoline exposure adversely affects multiple organs. Experimental evidence indicates that inhalation of gasoline fumes causes lung tissue injury, liver dysfunction, renal impairment, neurotoxicity, oxidative stress, and alterations in haematological parameters (Bokolo & Ligha, 2013; Elsayed et al., 2015; Uboh et al., 2022a). Similarly, prolonged gasoline exposure has been associated with abnormalities in bone marrow morphology and blood cell indices (Abubakar et al., 2015), while exposure to petroleum hydrocarbons has been reported to impair antioxidant defence mechanisms and promote lipid peroxidation (Oyebisi et al., 2013). Since bone is a metabolically active tissue that depends on

I. INTRODUCTION

Gasoline is one of the major refined products of crude oil and is extensively used as fuel for automobiles,

adequate mineralization, vascular supply, and hormonal regulation for normal growth and maintenance, chronic exposure to petroleum-derived toxicants may compromise skeletal integrity.

Evidence from related experimental studies suggests that petroleum products can adversely affect bone health. Mesembe et al. (2008) reported that maternal exposure to Bonny Light crude oil significantly reduced calcium, magnesium, copper, and manganese concentrations in the foetal femur, indicating impaired bone mineralization. Likewise, Garcia et al. (2015) demonstrated that chronic ethanol exposure reduced femoral cortical and trabecular bone thickness and mechanical strength, highlighting the susceptibility of bone tissue to toxic chemical exposure. Although these findings suggest that environmental toxicants can impair bone development and quality, they do not specifically explain how inhaled gasoline fumes affect the femoral bone.

Existing studies on gasoline toxicity have largely concentrated on oxidative stress, reproductive toxicity, haematological changes, hepatic injury, renal dysfunction, and neurological effects (Uboh et al., 2007; Abubakar et al., 2015; Uboh et al., 2022a). Comparatively few investigations have examined the direct effects of gasoline fume inhalation on bone tissue, particularly the femur, despite its importance as a major weight-bearing bone. More importantly, most previous studies used either a single sex or did not compare male and female responses, even though evidence indicates that biological sex may influence susceptibility to gasoline toxicity, with females exhibiting greater vulnerability in some physiological systems (Uboh et al., 2007). This leaves an important knowledge gap regarding whether inhaled gasoline fumes produce comparable or differential effects on femoral bone morphology and integrity in male and female Wistar rats.

Addressing this gap is important because sex-related differences in skeletal physiology, hormonal regulation, and bone metabolism may influence the extent of bone damage following toxic exposure. Understanding these differences could improve knowledge of petroleum-induced skeletal toxicity and provide useful experimental evidence for

occupational and environmental health risk assessment. Therefore, this study aims to comparatively evaluate the effects of inhaled gasoline fumes on the femoral bone of male and female Wistar rats, with a view to determining whether sex influences the skeletal response to chronic gasoline fume exposure.

Objectives

The aim of this study is to investigate comparative effects of inhaled gasoline fumes on the femoral bone of wistar rats of both sexes. Specifically the sought to

1. determine the effects of gasoline on bone using the femur bone of wistar rats
2. determine the effect of the inhaled gasoline on different sexes of wistar rats,

II. MATERIALS AND METHODS

Materials

The materials used for this study included gasoline, stainless steel cages, an exposure chamber, rat pellets, a one-litre can for gasoline administration, test animals, an electronic weighing balance, examination gloves, sawdust, surgical blades, and sample bottles for specimen collection.

Experimental Animals and Design

Fifty-two adult albino Wistar rats comprising twenty-six males and twenty-six females, weighing 135 ± 4 g, were obtained from the Animal House of the College of Medical Sciences, Imo State University, Owerri, Nigeria. The study was conducted from March to June 2021.

The rats were grouped into two major groups: the test group and the control group. Grouping was carried out according to the sexes of the animals. The exposure group was further divided into rats exposed to gasoline fumes for 6 hours (Group A) and rats exposed to gasoline fumes for 9 hours (Group B). Group A was subdivided into male (Group A1) and female (Group A2) rats, while Group B was subdivided into male (Group B1) and female (Group B2) rats. The control group (Group C) consisted of apparently healthy rats that did not receive any treatment.

The distribution of the rats is presented below.

Group	Exposure	Female	Male
Group A	6-hour gasoline exposure	7	7
Group B	9-hour gasoline exposure	6	6
Group C	Control	7	7

The rats were acclimatized in the experimental animal house for five days before commencement of the experiment. They were housed in stainless steel cages measuring 34 × 16 × 9 inches and fed normal rat pellets. All the rats were housed in a well-ventilated animal house and allowed free access to food and water throughout the experimental period. Animal care was carried out in accordance with the guidelines of the Institutional Animal Ethics Committee.

The test groups were exposed to gasoline fumes for 6 or 9 hours daily in the exposure chamber, while the control group was maintained in a gasoline fume-free section of the animal house.

Exposure to Gasoline Fumes

Petrol used for the study was purchased from the Empire Energy Filling Station near Imo State University, Owerri. The gasoline samples were stored in clean containers and kept in the laboratory until required for use.

The method of exposure described by Uboh et al. (2007) was adopted. The animal cages containing the test groups were placed inside an exposure chamber measuring 42 × 24 × 15 inches. One litre of gasoline was poured into two calibrated cans, each containing 0.16 litres of gasoline, and placed inside the chamber one hour before the commencement of exposure to allow the chamber to become saturated with gasoline vapour.

The exposed animals were then placed inside the chamber and allowed to inhale vapours generated by the direct evaporation of gasoline at ambient temperature and humidity. At the end of each daily exposure period, the rats were removed from the chamber. The same exposure procedure was maintained for several weeks. Animal care throughout the experiment complied with the guidelines of the Institutional Animal Ethics Committee.

Sample Collection

At the end of the experimental period, the animals were anaesthetized with chloroform and dissected for bone collection. The femur was harvested for histological study. The harvested bones were fixed in 10% formal saline and transported to the University of Port Harcourt Teaching Hospital for tissue processing and histological examination. The histochemical technique employed was Haematoxylin and Eosin (H&E) staining.

Histological Techniques

Tissue Processing

The animals were sacrificed, and the required organs were harvested and sent to the histology laboratory for processing.

Gross Examination

Thin tissues were selected from the bones, muscles, brain, spinal cord, and vertebrae. Soft tissues were processed accordingly, while the bony tissues were first decalcified before processing.

Decalcification and Tissue Processing

Bony tissues measuring approximately 2 mm thick were decalcified using 10% formal saline solution for three days. The endpoint of decalcification was determined using ammoniacal solution.

The tissues were subsequently dehydrated in 70%, 95%, and absolute alcohol in two changes of two hours each. The dehydrated tissues were cleared in xylene in two changes of two hours each and impregnated in molten paraffin wax in two changes of two hours each. The tissues were embedded in paraffin wax, allowed to solidify, and removed from the embedding mould. The paraffin blocks were trimmed, exposed to an ice block, sectioned at 5 µm, floated on a warm water bath, mounted on clean glass slides, labelled, and allowed to drain and dry.

Haematoxylin and Eosin Staining

The tissue sections were dewaxed in xylene in two changes for 30 minutes to one hour and rehydrated through absolute alcohol, 95% alcohol, and 70%

alcohol before washing in water. The sections were stained in Ehrlich's haematoxylin for 30 minutes, washed in water, differentiated briefly in 1% acid alcohol, washed again, and blued in Scott's tap water for 10–20 minutes. Following another wash, the sections were counterstained in eosin solution for 1

minute, dehydrated through ascending grades of alcohol, cleared in xylene, mounted with DPX mounting medium using clean cover slips, labelled, and examined microscopically using a $\times 10$ objective with photomicrograph facilities

III. RESULTS

Table 1. Distribution of Wistar Rats According to Experimental Groups

Parameters	Group A (6-h Exposure)	Group B (9-h Exposure)	Group C (Control)
Duration of gasoline fume exposure (hours/day)	6	9	Nil
Number of female rats	7	6	7
Number of male rats	7	6	7
Total number of rats	14	12	14
Experimental condition	Gasoline exposure	Gasoline exposure	No gasoline exposure

The rats in Groups A and B constituted the test groups and comprised both male and female Wistar rats exposed to gasoline fumes in an exposure chamber for different daily durations of 6 hours and 9 hours, respectively. Group C served as the control group and

consisted of male and female rats that were not exposed to gasoline fumes throughout the experimental period. Table 1 presents the distribution of the rats according to experimental group, sex, and duration of gasoline fume exposure.

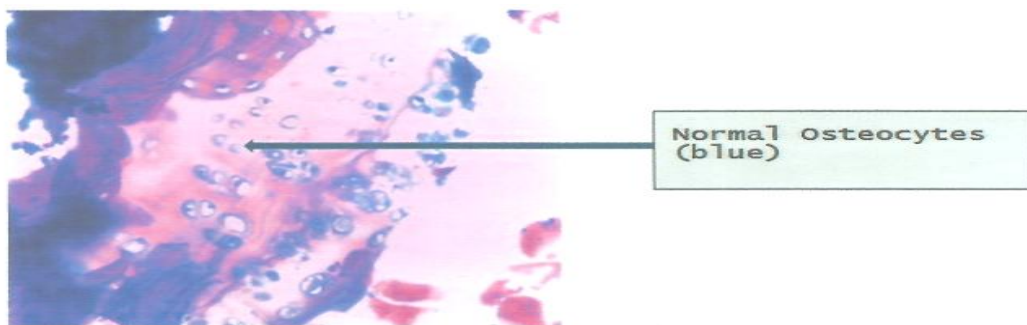


Fig. 1: Micrograph of the Femur Bone Tissues of Female Rats exposed to Gasoline Fumes for 9 hours

In fig. 1 above, there was no abnormality in the histo-architecture of the femur bone of the female rats after exposure to gasoline for 90 days. The micrograph indicates that the osteocytes appeared normal.

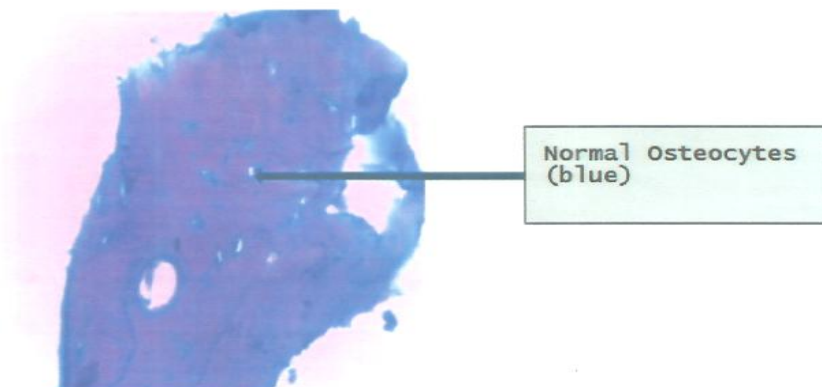


Fig. 2: Micrograph of the Femur Bone Tissues of Male Rats exposed to Gasoline Fumes for 9 hours

In fig. 2 above, there was no abnormality in the histo-architecture of the femur bone of the male rats after exposure to gasoline for 90 days. The micrograph indicates that the osteocytes appeared normal.



Fig. 3: Micrograph of the Femur Bone Tissues of Male Rats exposed to Gasoline Fumes for 6 hours

In fig 3 above, there was no abnormality in the histo-architecture of the femur bone of the male rats after exposure to gasoline for 90 days. The micrograph indicates that the osteocytes appeared normal

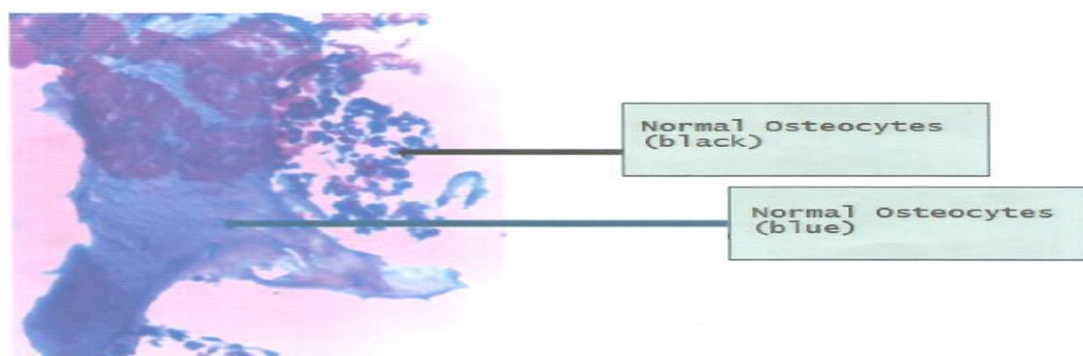


Fig. 4: Micrograph of the Femur Bone Tissues of Female Rats exposed to Gasoline Fumes for 6 hours

Fig. 4 above, there was no abnormality in the histo-architecture of the femur bone of the female rats after exposure to gasoline for 90 days. The micrograph indicates that the osteocytes appeared normal with blue and black colorations.

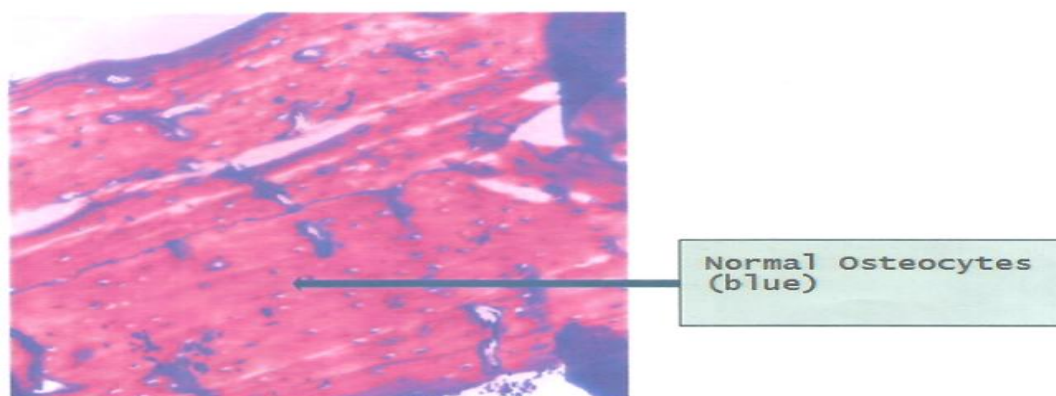


Fig. 5: Micrograph of the Femur Bone Tissues of Female Rats that were not exposed to any Substance (control)

In fig. 5 above, there was no abnormality in the histo-architecture of the femur bone of the female rats that were not exposed to either gasoline or prednisolone. The micrograph indicates that osteocytes appeared normal

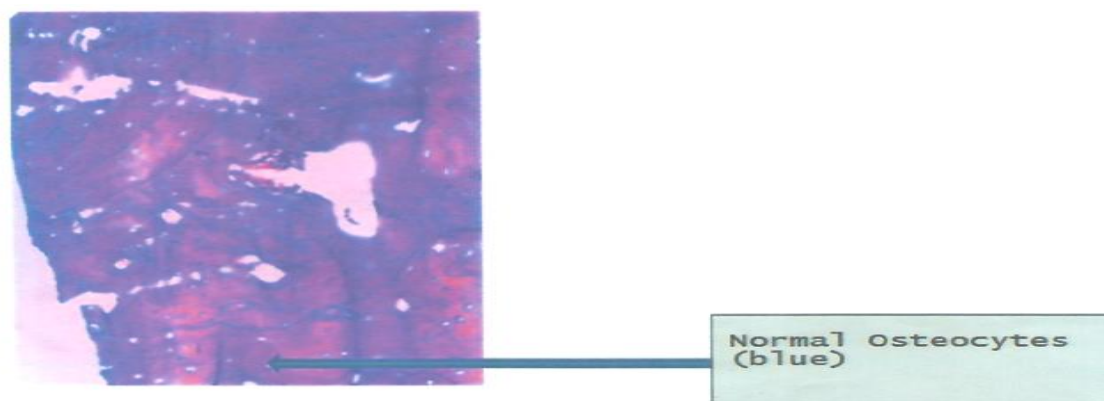


Fig. 6: Micrograph of the Femur Bone Tissues of Male Rats that were not exposed to any Substance (control)

In Fig. 6 above, there was no abnormality in the histo-architecture of the femur bone of the male rats that were not exposed to gasoline. The micrograph indicates that osteocytes appeared normal.

IV. DISCUSSION

The present study investigated the effects of inhaled gasoline fumes on the femoral bone of Wistar rats exposed for 6 and 9 hours daily. Histological examination revealed no marked alterations in bone cells or femoral histoarchitecture, as the trabecular architecture, osteocytes, and overall bone morphology remained comparable to those of the control group. This suggests that gasoline fume inhalation under the experimental conditions did not produce detectable femoral bone damage. The absence of histological abnormalities may be attributed to the use of unleaded gasoline, which contains lower lead concentrations and, therefore, may have reduced skeletal toxicity.

The findings differ from those of Abubakar et al. (2015), who reported alterations in blood cell indices and bone marrow morphology following 11 weeks of gasoline vapour exposure. The discrepancy may be due to differences in the tissues examined and the longer exposure duration in their study. Similarly, the present results contrast with Garcia et al. (2015), who found that chronic ethanol exposure reduced femoral bone thickness and mechanical strength, and with Mesembe et al. (2008), who reported impaired foetal femur mineralization following maternal crude oil exposure. These differences may be explained by

variations in the toxic agents, routes of exposure, developmental stages, and study endpoints.

The study also compared the effects of gasoline fumes on male and female Wistar rats and found no observable sex-related differences in femoral bone histoarchitecture after exposure for either 6 or 9 hours daily. Both sexes maintained normal trabecular organization and bone cellular morphology comparable to the control group, indicating similar skeletal responses to gasoline inhalation. This finding contrasts with Uboh et al. (2007), who reported greater susceptibility in female rats based on reproductive hormonal changes. The disparity is likely due to differences in the parameters assessed, as hormonal responses are inherently sex-dependent, whereas the present study focused on bone histology. Likewise, although Uboh et al. (2022a) demonstrated that gasoline exposure induced oxidative stress through increased BTEX accumulation and reduced antioxidant enzyme activity, the present study found no corresponding femoral bone histopathological changes. This shows that biochemical evidence of oxidative stress may not necessarily result in detectable structural alterations in bone within the exposure duration used in this study.

V. CONCLUSION

Based on the histological findings, inhalation of gasoline fumes did not produce observable alterations in the femoral bone histoarchitecture of Wistar rats under the conditions of this study. Examination of the femoral bone sections from rats

exposed to gasoline fumes for 90 days revealed normal bone architecture, with osteocytes maintaining their normal morphology in both the 6-hour and 9-hour exposure groups. Similarly, the control animals exhibited normal femoral bone histology.

Furthermore, comparison between male and female Wistar rats showed no apparent sex-related differences in the histological response to gasoline fume inhalation. Both sexes demonstrated preserved femoral bone architecture and normal osteocyte appearance following exposure.

The findings revealed that prolonged inhalation of gasoline fumes, as administered in this study, did not induce detectable histopathological changes in the femoral bone of either male or female Wistar rats. These results indicate that, under the exposure conditions employed, gasoline fume inhalation had no observable adverse effect on the femoral bone histoarchitecture of Wistar rats of both sexes.

VI. RECOMMENDATIONS

1. Further studies should evaluate the effects of longer exposure periods and different concentrations of gasoline fumes on the femoral bone.
2. Future research should employ additional techniques, such as bone mineral density and biochemical analyses, to detect subtle bone changes.
3. Studies with larger sample sizes and different age groups of Wistar rats are recommended to validate the findings.
4. Further comparative studies should be conducted to confirm the effects of gasoline fumes on the femoral bone of male and female Wistar rats.

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