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Biochemical and and Lipid Profile Alterations in Wistar Rats Following Toluene Exposure

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Abstract

The volatile organic compound toluene is known for its toxicity to humans. This study aimed to evaluate its effects on lipid profile and certain biochemical markers in Wistar rats. Forty adult female Wistar rats were distributed across ten groups (four rats each). Group 1 received distilled water, while groups 2 to 9 were administered increasing doses of analytical grade toluene over a 28-day period. Unfortunately, animals in groups 6 to 10 succumbed during the study, while those in groups 1 to 5 were euthanized after 28 days under chloroform anesthesia for blood sample collection via cardiac puncture. The results indicated a significant elevation ($p < 0.05$) in serum levels of triglycerides, total bilirubin, low-density lipoprotein, and total cholesterol following toluene exposure. Conversely, concentrations of high-density lipoprotein, albumin, and total protein were significantly reduced ($p< 0.05$). These findings suggest that toluene adversely affects lipid profile, total protein, albumin, and total bilirubin, potentially through its systemic influence on oxidative stress, inflammation, and liver function.

Keywords: High-density lipoprotein, Toluene, Total Bilirubin, Total Protein, Bilirubin, Triglycerides, Total Cholesterol, Low-density Lipoprotein.

Introduction

Toluene (also known as methylbenzene or phenylmethane) is a highly volatile organic solvent commonly utilized in various industrial processes, such as plastic production, petroleum refining and chemical synthesis (Cruz et al., 2014; Park et al., 2022). Its high volatility makes inhalation the primary exposure route, while its lipid solubility enables additional absorption through skin contact, creating dual exposure risks in occupational settings (Soares et al., 2020). Chronic exposure even at low concentrations can lead to significant toxicological effects due to the compound's ability to accumulate in biological systems and disrupt normal physiological functions over time (Holmes et al., 2024; Okas et al., 2023; Handlos et al., 2022).

Beyond industrial hazards, toluene's abuse potential arises from deliberate inhalation of vapours, either from pure sources or household products like paints, adhesives and inks (Cruz et al., 2014; Handlos et al., 2022; Heeley-Hill, et al., 2021). Though its precise mechanism remains unclear, toluene modulates diverse ion channels (voltage-gated $Na^+/K^+/Ca^{2+}$ and ligand-gated $GABA_a$/NMDA/nACh receptors), contributing to its neurotoxic and psychoactive effects (Cruz et al., 2014).

Current studies prioritize acute toxicity given its common occupational hazards and immediate physiological impacts (Benson et al., 2021; El-Hagrasy et al., 2025; Rajput et al., 2025). Despite documented neurotoxicity, toluene's presence in consumer products contributes to its underestimated hazard potential among recreational solvent users, leading to recurrent poisonings (Hubková et al., 2022; Pal et al., 2023; Kamani et al., 2023). For instance, a single emergency department reported 20 toluene intoxication cases biannually (Holmes & Murray, 2024) likely a gross underestimate as stigma and mild symptoms deter many exposed individuals from seeking medical attention (Khadhar et al., 2024).

Acute exposure carries a 15% mortality rate, though epidemiological data are limited. While early intervention often prevents long-term effects, severe manifestations (coma, refractory acidosis, electrolyte disturbances) predict poor outcomes (Holmes & Murray, 2024). Multiorgan failure (ARDS, lethal arrhythmias, and acute renal injury) all contribute to its significant mortality risk (Maruniak et al., 2025; Schwarz et al., 2021). In this study, therefore, the effect of increasing doses of toluene on lipid profile and other important biochemical parameters was investigated. This is with the view of documenting safety levels since its exposure by humans and animals is inevitable in a society that is not completely free from the products of toluene.

Methodology

A total of 40 healthy female Wistar albino rats (150–200 g) were obtained from the animal facility at the University of Port Harcourt's Department of Pharmacology. They were then transferred and maintained at Niger Delta University's Faculty of Basic Medical Sciences under controlled laboratory conditions, including a 12-hour light/dark cycle, regulated ventilation and unrestricted access to commercial feed (Delta Feeds grower's mash) and water. The animals underwent a 14-day acclimatization period before experimentation

Experimental Design

The study employed 40 rats equally distributed across 10 experimental groups, receiving daily oral gavage treatments for 28 consecutive days.

Group 1: Normal control: distilled water.
Group 2: 0.125ml/kg body weight.
Group 3: 0.25ml/kg body weight.
Group 4: 0.5ml/kg body weight.
Group 5: 1ml/kg body weight.
Group 6: 3ml/kg body weight.
Group 7: 4ml/kg body weight.
Group 8: 5ml/kg body weight.
Group 9: 6ml/kg body weight.
Group 10: 7ml/kg body weight.

Following the 28-day experimental period, all subjects were euthanized under chloroform-induced anesthesia.

Collection of Samples

Blood was collected through cardiac puncture into non-anticoagulant tubes, allowed to coagulate for 30 minutes and subsequently centrifuged at 2000 revolutions per minute for 10 minutes to isolate serum for biochemical analysis.

Determination of Biochemical Parameters

Biochemical parameters including serum proteins (total protein, albumin), bilirubin and lipid profiles (triglycerides, HDL, total cholesterol, LDL) were measured spectrophotometrically using Randox assay kits following manufacturer protocols.

Statistical Analysis

Data are presented as mean ± SD. Statistical significance was determined using one-way ANOVA in SPSS version 10.0 software, adopting $p<0.05$ as the significance level.

Results

The results from this study are expressed in the bar charts below.

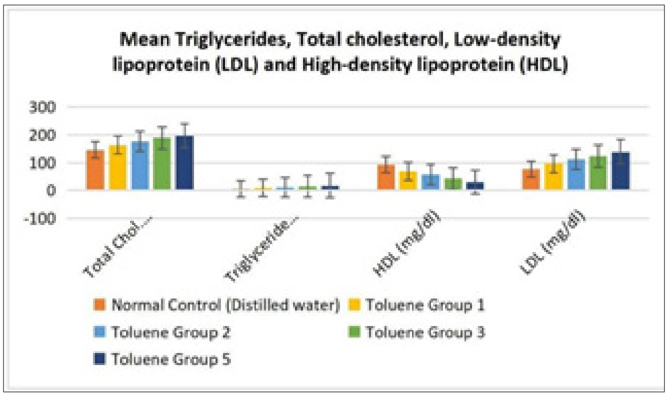

Figure 1: The effects of toluene on serum concentrations of Triglycerides, total cholesterol, low-density lipoprotein and high-density lipoprotein (HDL) in toluene treated Wistar rats.

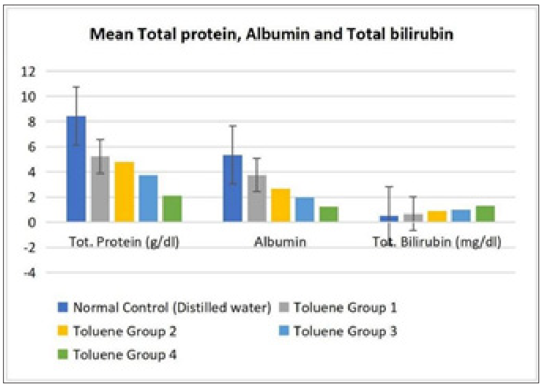

Figure 2: The effects of toluene on serum concentrations of Total protein, Albumin and Total bilirubin in toluene treated Wistar rats.

Discussion

BTEX (benzene, toluene, ethylbenzene, and xylenes) represent the primary toxic constituents found in gasoline formulations, posing significant health and environmental risks (Muda et al., 2024; Anigilaje et al., 2024). As volatile organic compounds (VOCs), BTEX components are pollutants commonly found at low concentrations in the air, water and soil (David & Niculescu, 2021). According to the International Agency for Research on Cancer (IARC, 2012) toluene has been categorized as a potential human carcinogen, emphasizing its hazardous nature as a toxic volatile organic compound within the BTEX chemical group. IARC's classification stems from toluene's established association with acute myeloid leukemia and non-lymphocytic leukemia, with multiple recent meta-analyses confirming its connection to various hematological cancers through occupational exposure data (Saeedi et al., 2024). The 2018 IARC monograph maintained toluene's Group 1 classification, citing substantial evidence of multiorgan carcinogenicity in animal studies (International Agency for Research on Cancer [IARC], 2018). This carcinogenic potential arises from toluene's metabolic conversion to reactive intermediates that generate oxidative stress through elevated ROS production (Setya-Maryiantari et al., 2025; Zhou et al., 2024).

This study aimed to evaluate the lipid profile and certain biochemical changes (including total protein, albumin, and total bilirubin) in Wistar rats exposed to varying concentrations of toluene over twentyeight days. Unfortunately, all animals in groups 6-10 died early in the experiment, while those in groups 1 to 5 were euthanized at the end of twentyeight days for biochemical analyses.

The results revealed that administration of serial concentrations of toluene caused a dose-dependent elevation ($p<0.05$) in serum triglycerides, total cholesterol and LDL levels, while significantly reducing HDL concentrations. This suggests that toluene exposure may indirectly affect lipid metabolism through its broader systemic effects on the body. Previous study links chronic toluene exposure to oxidative stress and inflammatory responses that could alter lipid homeostasis through peroxidative pathways (Shin et al., 2022).

Additionally, toluene exposure has been linked to liver damage. Given the liver's pivotal role in lipid metabolism, any impairment caused by toluene could disrupt lipid metabolism, resulting in abnormalities in lipid profile parameters such as cholesterol and triglyceride levels (Malaguarnera et al., 2012; Brauner et al., 2020; Pei et al., 2020). Although there is limited direct research on toluene's effects on lipid metabolism, it is conceivable that its systemic effects on oxidative stress, inflammation and liver function could indirectly influence lipid profile. However, further research is necessary to fully elucidate these mechanisms and their extent.

The study also investigated toluene exposure's effects on serum total protein, albumin and total bilirubin. Results showed a dose-dependent significant ($p<0.05$) decrease in serum total protein and albumin levels, alongside a dose-dependent significant increase ($p < 0.05$) in serum total bilirubin concentration, likely due to toluene's toxic impact on the liver, consistent with previous findings (Malaguarnera et al., 2012; Umicevic et al., 2022).

Conclusion

In conclusion, the study's findings suggest that toluene exposure negatively impacts lipid profile, total bilirubin, albumin and total protein levels in the body. Further research is warranted to elucidate toluene's mechanisms of toxicity.

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