SIAR - Global Journal of Medicine & Pharmaceutical Review (GJMPR)
www.siarpublications.org
info@siarpublications.org
ISSN: 3122-0762
Vol. 1 Issue 1 July-Aug. 2025

Toxicological Evaluation of Hepatic, Renal and Oxidative Stress Parameters in Xylene Treated Wistar Rats

Ezomoh Olusoga Olubunmi[1], Chukwuma Samuel Anakwe[2], Ogu Oyinbrakemi Collins[3], Ogbara Kievelezebo Peter[4] and Prohp The Prophet[5]

Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

Abstract

This study investigated the dose-dependent toxicological effects of subacute xylene exposure on hepatic, renal, and oxidative stress parameters in Wistar rats. Forty adult male rats were divided into ten groups (n=6). The normal control group (Group 1) received distilled water, while Groups 2 to 10 were administered increasing doses of xylene (0.125 to (7 mL/kg) body weight) via oral gavage for 28 days. Significant mortality was observed, with all animals in groups receiving ≥ (3 mL/kg) succumbing before the study concluded, limiting biochemical analysis to Groups 1-5 (0-(1 mL/kg)). Xylene exposure induced severe, dose-dependent hepatotoxicity, evidenced by significant elevation in serum ALT, AST, ALP, and total bilirubin, alongside decreased total protein and albumin. Nephrotoxicity was indicated by increased urea and creatinine levels and a significant disruption in electrolyte and acid-base homeostasis. Mechanistically, a significant depletion of antioxidant enzymes (SOD, CAT, GPx) and a rise in lipid peroxidation (MDA) were observed in both liver and kidney tissues, implicating oxidative stress as a central pathway of toxicity. The results provide evidence that xylene induces multi-organ toxicity primarily through an oxidative mechanism, culminating in organ failure and mortality at higher doses. These findings highlight a substantial occupational health risk and underscore the need for stringent exposure control measures.

KEYWORDS: Antioxidants, Hepatotoxicity, Lipid peroxidation, Renal toxicity, Xylene.

Introduction

Industrialization has brought about significant advancements in human society, but it has also introduced a myriad of chemical hazards into occupational and environmental settings. Among these, organic solvents represent a major class of chemicals widely used across various industries, including paint and coating manufacturing, printing, rubber and leather production, laboratory work, and the pharmaceutical industry (Verma et al., 2022). Xylene, an aromatic hydrocarbon, is one of the most prevalent high-production-volume solvents globally due to its excellent dissolving properties and effectiveness as a cleaning and degreasing agent (Saggu and Kumar, 2025). Chemically, it exists as a mixture of three isomers (ortho-, meta-, and para-xylene) and is often used in conjunction with other solvents like toluene and benzene (Dural et al., 2025).

The widespread use of xylene necessitates a critical examination of its potential health impacts. Human exposure, primarily through inhalation of vapors and, to a lesser extent, dermal contact, is a significant concern for workers in these industries. Furthermore, environmental release and subsequent contamination of air and groundwater pose a risk to the general population living near industrial areas (Niaz *et al*., 2015; Shojaee Barjoee *et al*., 2021). The volatility of xylene ensures

that inhalation remains the primary route of exposure, leading to systemic absorption and distribution to highly perfused organs (Niaz *et al*., 2015). Upon absorption, xylene is rapidly distributed throughout the body. It is primarily metabolized in the liver by the cytochrome P450 enzyme system, specifically CYP2E1, into its main metabolite, methylhippuric acid, which is excreted in urine (Soleimani, 2020). This metabolic pathway, while a detoxification mechanism, can also be a source of toxicity. The bioactivation process can generate reactive oxygen species (ROS), leading to oxidative stress, a key mediator of chemical-induced cellular damage (D'Souza *et al*., 2024). The liver, as the primary site of metabolism, and the kidneys, as the principal organs of excretion, are particularly vulnerable to the toxic effects of xylene and its metabolites (Soleimani, 2020; Candura *et al*., 2020).

Initial symptoms of acute xylene exposure in humans are predominantly neurological, including dizziness, headache, nausea, and impaired coordination (Faulhammer *et al*., 2024). However, the insidious nature of repeated, low-level occupational exposure often leads to subclinical damage that may go unnoticed until significant pathological changes occur in vital organs.

The liver and kidneys are indispensable for maintaining metabolic homeostasis and are frequent targets for xenobiotic-induced injury (Rad *et al*., 2024). The liver's central role in the biotransformation of xenobiotics makes it highly susceptible to toxic insult. Hepatotoxicity can manifest as oxidative stress, inflammation, fatty changes (steatosis), necrosis, and altered liver function tests, including elevated levels of enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) (Tamber *et al*., 2023).

Concurrently, the kidneys, responsible for filtering blood and concentrating waste products for excretion, are exposed to high levels of xenobiotics and their metabolites (Yameny, 2025). Nephrotoxicity can result in impaired renal function, indicated by elevated levels of biomarkers such as serum creatinine, blood urea nitrogen (BUN), and uric acid, as well as histopathological alterations in the glomeruli and tubules (Kalamkar *et al*., 2024). The concurrent evaluation of hepatic and renal parameters provides a comprehensive assessment of the systemic toxicological impact of a compound like xylene.
A growing body of evidence suggests that the toxicity of many organic solvents, including xylene, is intricately linked to the generation of oxidative stress (Suaidi *et al*., 2024). This state arises from an imbalance between the production of ROS (such as superoxide anion, hydrogen peroxide, and hydroxyl radicals) and the body's antioxidant defense capacity. Key enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), while non-enzymatic antioxidants include reduced glutathione (GSH) and vitamins C and E (Bandodkar *et al*., 2025).

An overabundance of ROS can overwhelm these defenses, leading to lipid peroxidation of cellular membranes (measured by malondialdehyde, MDA, levels), oxidative damage to proteins and DNA, inflammation, and ultimately, cell death via apoptosis or necrosis (Bellanti *et al*., 2025). This mechanism is a proposed pathway for xylene-induced damage to the liver and kidneys, making the assessment of oxidative stress markers a crucial component of its toxicological profile. Despite its widespread use, a comprehensive toxicological profile of xylene that integrates hepatic, renal, and oxidative stress parameters in a single in vivo study remains somewhat underexplored, particularly concerning sub-chronic exposure scenarios relevant to occupational settings. While previous studies have hinted at its hepatotoxic and nephrotoxic potential, a simultaneous and

detailed evaluation of functional biomarkers, histopathological changes, and the underlying oxidative stress mechanism is warranted.

Chemical/ Reagents

Chloroform, xylene (JDH, Bangladesh). Biochemical kits (Total protein) (Randox LTD., United Kingdom), elisa immunoassay kits for sex hormones (Elabscience Biotechnology Co., Ltd., Germany)

Experimental Design

The study was conducted using sixty rats, which were evenly divided into ten experimental groups. Each group received daily oral gavage treatments for a period of 28 consecutive days. The normal control group (Group 1) was administered distilled water, while Groups 2 through 10 were treated with increasing doses of the test substance, ranging from (0.125 mL/kg) body weight in Group 2 to (7 mL/kg) body weight in Group 10. Specifically, the doses administered were 0.125, 0.25, 0.5, 1, 3, 4, 5, 6, and (7 mL/kg) body weight for Groups 2 to 10, respectively. At the end of the 28-day experimental period, all animals were humanely sacrificed after anesthesia induced by chloroform inhalation.

Collection of Samples

Blood samples were obtained via cardiac puncture and transferred into plain (non-anticoagulant) tubes. The samples were left undisturbed for 30 minutes to allow clot formation, after which they were centrifuged at 2000 rpm for 10 minutes to separate the serum for subsequent biochemical analysis.

Determination of Biochemical Parameters

Serum biochemical markers, such as AST, ALT, ALP, total protein, albumin, bilirubin, urea, and creatinine, were quantified spectrophotometrically using Randox diagnostic kits in accordance with the manufacturer's instructions.

Determination of Oxidative Stress Parameters

Superoxide dismutase (SOD) was assayed by the method described by Marklund and Marklund (1974), Catalase (CAT) activity was estimated according to the method of Aebi *et al*., (1974), glutathione peroxidase (GPx) activity was estimated using a coupled enzyme assay system, which was connected to glutathione reductase (GR), according to the method described by Lawrence and Burk (1976) while lipid peroxidation was estimated as malondialdehyde (MDA) according to the method described by Armstrong and Al-Awadi (1991).

STATISTICAL ANALYSIS

All the results were expressed as Mean ± Standard deviation. The statistical significance was evaluated using the One-way Analysis of Variance (ANOVA) (SPSS 25.0). Statistical significance was set at ($p<0.05$).

()Ethical Approval

()The Research and Ethics Committee of the College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria granted ethical approval for this study.

Results

Tragically, the groups administered 3, 4, 5, 6 and (7 mL/kg) xylene respectively, died midway in the experimentation period. The tables below show results for control and groups administered 0.125, 0.25, 0.5 and 9 (1 mL/kg)) xylene respectively.

Table 1. Serum level of hepatic biomarkers in Wistar rats treated with xylene

Experimental Group	ALT (U/L)	AST (U/L)	Total Protein (g/dl)	Albumin (g/dl)	Total Bilirubin (mg/dl)	ALP (U/L)
Distilled water	71.22±3.02^{a}	75.85±2.86^{a}	8.22±1.07^{a}	4.89±0.65^{a}	0.47±0.03^{a}	76.38±4.20^{a}
Xylene (1.125 mL/kg)	91.37±3.95^{b}	120.54±4.65^{b}	5.31±1.08^{b}	3.54±0.11^{b}	0.71±0.03^{b}	95.48±5.06^{b}
Xylene (0.25 mL/kg)	117.87±6.03^{c}	134.23±4.13^{c}	4.06±0.87^{c}	2.72±0.04^{c}	0.84±0.04^{c}	105.87±5.88^{c}
Xylene (0.5 mL/kg)	124.45±5.85^{d}	143.78±7.28^{d}	3.68±0.65^{d}	1.98±0.04 ^{d}	0.95±0.03 ^{d}	130.56±7.03^{d}
Xylene (1 mL/kg)	142.65±5.24^{e}	149.87±4.22^{e}	2.32±0.05^{e}	1.34±0.15^{e}	1.21±0.05^{e}	146.72±5.98^{e}

Data are expressed as the (mean ± SD) (n = 6). Means within the same column carrying different superscripts are significantly ($p < 0.05$)) different.

Table 2. Serum level of renal biomarkers in Wistar rats treated with xylene

Experimental Group	Urea (mg/dl)	Creatinine (mg/dl)	Sodium (mEq/Ll)	Potassium (mEq/L)	Chloride (mEq/L)	Bicarbonate (mol/L)
Distilled water	60.88±6.11^{a}	0.60±0.02 ^{a}	69.67±4.85^{a}	1.78±0.08 ^{a}	24.78±3.16 ^{a}	39.78±3.72 ^{a}
Xylene (0.125 mL/kg))	77.63±5.04^{b}	0.76±0.03 ^{b}	75.98±4.03^{b}	2.05±0.05 ^{b}	29.90±4.14 ^{b}	33.45±2.48 ^{b}
Xylene ((0.25 mL/kg))	88.96±5.63^{c}	0.97±0.04^{c}	92.78±6.22^{c}	2.80±0.08^{c}	45.84±3.88 ^{c}	25.08±4.2 ^{c}
Xylene ((0.5 mL/kg))	100.56±4.75 ^{d}	1.13±0.03 ^{d}	101.54±5.08 ^{d}	3.10±0.12 ^{d}	53.98±5.34 ^{d}	13.98±1.74 ^{d}
Xylene (1 mL/kg))	120.38±7.43 ^{e}	1.23±0.02 ^{e}	120.85±7.85 ^{e}	4.05±0.06 ^{e}	68.68±3.75 ^{e}	6.43±1.58^{e}

Data are expressed as the (mean ± SD) (n = 6). Means within the same column carrying different superscripts are significantly ($p < 0.05$)) different.]

Table 3. Liver tissue antioxidant profile in Wistar rats treated with xylene

Experimental Group	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	MDA (U/mg protein)
Distilled water	9.32±1.18[a]	7.86±1.62[a]	8.30±1.22[a]	2.42±0.07[a]
Xylene (0.125 mL/kg))	6.79±1.31[b]	5.87±0.71[b]	7.02±1.43[b]	4.94±0.42[b]
Xylene (0.25 mL/kg))	5.02±0.48[c]	4.12±0.49[c]	5.86±1.18[c]	6.38±0.74[c]
Xylene (0.5 mL/kg))	3.87±0.36[d]	3.32±0.12[d]	3.94±0.17[d]	7.89±0.38[d]
Xylene (1 mL/kg))	2.28±0.09 [e]	2.34±0.41[e]	2.08±0.15[e]	9.90±0.44[e]

Data are expressed as the (mean ± SD) (n = 6). Means within the same column carrying different superscripts are significantly ($p < 0.05$)) different.

Table 4. Kidney tissue antioxidant profile in Wistar rats treated with xylene

Experimental Group	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	MDA (U/mg protein)
Distilled water	10.23±1.87[a]	7.95±1.35[a]	8.08±0.86[a]	2.11±0.52[a]
Xylene ((0.125 mL/kg))	7.23±1.75[b]	5.89±0.32[b]	6.42±0.53[b]	4.76±0.38[b]
Xylene (0.25 mL/kg))	6.80±2.01[c]	4.02±0.63[c]	4.87±0.44[c]	6.23±0.68[c]
Xylene (0.5 mL/kg))	4.78±0.82[d]	3.98±0.74[d]	3.87±0.63[d]	7.85±1.44[d]
Xylene (1 mL/kg))	2.10±0.52[e]	2.07±0.22[e]	2.93±0.74[e]	9.67±1.31[e]

Data are expressed as the (mean ± SD) (n = 6. Means within the same column carrying different superscripts are significantly ($p < 0.05$)) different.

Discussion

Scientific Discussion

The results of this study provide a comprehensive and unequivocal demonstration of the multi-organ toxicological impact of xylene exposure in a Wistar rat model. The data reveal a consistent pattern of severe, dose-dependent damage to both the hepatic and renal systems, with oxidative stress identified as a central mechanistic pathway. These findings align with and significantly extend the existing body of literature on hydrocarbon toxicity.

The observed alterations in serum biomarkers present a classic picture of mixed hepatocellular and cholestatic liver injury. The dose-dependent, statistically significant elevation in ALT, AST, and ALP is a hallmark of xenobiotic-induced hepatocyte necrosis and biliary tract damage (Suresh *et al*., 2025). Our findings are consistent with previous work by (Abouee-Mehrizi *et al*. (2021), who reported similar increases in liver enzymes in rats exposed to toluene, a related aromatic hydrocarbon. The concurrent, dose-dependent decline in total protein and albumin levels indicates

a profound impairment of the liver's synthetic function, a sign of advanced hepatotoxicity that moves beyond mere cellular leakage to functional failure (Verzár *et al*., 2025).

Notably, this study provides robust parallel evidence of significant nephrotoxicity. The dramatic rise in urea and creatinine levels across xylene groups indicates a severe impairment of glomerular filtration rate (GFR), a primary marker of renal dysfunction (Peres & da Cunha, Boukarine *et al*., 2023). The electrolyte imbalances, hyperkalemia, hypernatremia, hyperchloremia, and decreased bicarbonate, paint a picture of compromised renal tubular function, affecting both electrolyte reabsorption and acid-base homeostasis. This pattern of renal injury aligns with the findings of **Airhomwanbor *et al*. (2024)**, who documented similar renal impairment in petrol attendants working in petrol stations, suggesting a common toxic pathway for certain hydrocarbons.

The most compelling evidence from this study lies in the clear dose-response relationship in the oxidative stress markers across both liver and kidney tissues. The significant depletion of key antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), coupled with a concomitant rise in malondialdehyde (MDA), a marker of lipid peroxidation, provides irrefutable evidence that oxidative stress is a primary mechanism of xylene-induced toxicity.

This finding strongly supports and expands upon the work of (Uboh, et a., **2022)**, who specifically demonstrated xylene-induced lipid peroxidation in the rat liver. Our study confirms their findings and crucially demonstrates that this oxidative assault is not organ-specific but is systemic, affecting the kidneys with equal severity. The depletion of antioxidant defenses suggests that xylene metabolites overwhelm the endogenous protective systems, leading to a cascade of cellular damage. The resulting lipid peroxidation disrupts the integrity of cellular and organellar membranes, which directly explains the leakage of enzymes from hepatocytes (elevated ALT/AST) and the loss of functional capacity in renal tubules and hepatocytes.

Conclusion

This study demonstrates that xylene exposure induces severe, dose-dependent hepato-nephrotoxicity mediated primarily through oxidative stress. The results underscore a significant public health risk, particularly for individuals with occupational exposure to xylene. These findings strongly advocate for the reinforcement of safety protocols and suggest that therapeutic strategies aimed at boosting antioxidant defenses (antioxidant supplementation) could be a valuable area for future research to mitigate the toxic effects of such exposures.

Conflict of interest

There is no conflict of interest

Acknowledgement

We wish to thank Rebecca Ogoriba Eferebo for providing us wit htechnical support.

References

Abouee-Mehrizi, A., Rasoulzadeh, Y., Mehdipour, A., Alihemmati, A., & Rahimi, E. (2021). Hepatotoxic effects caused by simultaneous exposure to noise and toluene in New Zealand white rabbits: a biochemical and histopathological study. *Ecotoxicology*, *30*(1), 154-163.

Airhomwanbor, K. O., Oweifa, J. T., Obohwemu, K. O., & Bello, G. O. (2024). Effect of petrochemicals to the renal electrolytes of fuel attendants (a case study of Oluyole area in Ibadan, Nigeria). *Acta Scientific MEDICAL SCIENCES (ISSN: 2582-0931)*, *8*(10).

Bandodkar, V. V., Moger, V. S., & Baliga, P. (2025). Antioxidant Defense Mechanisms: Enzymatic and Non-Enzymatic. In *The Role of Reactive Oxygen Species in Human Health and Disease* (pp. 43-80). IGI Global Scientific Publishing.

Bellanti, F., Coda, A. R. D., Trecca, M. I., Buglio, A. L., Serviddio, G., & Vendemiale, G. (2025). Redox Imbalance in Inflammation: The Interplay of Oxidative and Reductive Stress. *Antioxidants*, *14*(6), 656.

Boukarine, R., Hamdi, L., & Khelili, K. (2023). Therapeutic Effects of Rocket Leaves (Eruca sativa) against Hepato-Renal Toxicity Caused by Xylene in Wistar Rats. *Pakistan Journal of Zoology*, *55*(3), 1437.

Candura, S. M., Manzo, L., Castoldi, A. F., & Costa, L. G. (2020). Metabolism and toxicity of occupational neurotoxicants: genetic, physiological, and environmental determinants. In *Occupational neurotoxicology* (pp. 21-48). CRC Press.

D'Souza, L. C., Paithankar, J. G., Stopper, H., Pandey, A., & Sharma, A. (2024). Environmental chemical-induced reactive oxygen species generation and immunotoxicity: a comprehensive review. *Antioxidants & Redox Signaling*, *40*(10-12), 691-714.

Dural, E., Kaya, B. İ., Mergen, G., Boran, E., & Söylemezoğlu, T. (2025). Determination of benzene, toluene, ethylbenzene, p-, m-, o-xylene, and n-butyl acetate in urine by a validated gas chromatography method: Application to an occupational monitoring study. *Brazilian Journal of Pharmaceutical Sciences*, *61*, e231017.

Faulhammer, F., Rooseboom, M., Kocabas, N. A., Arts, J. H., Cordova, A., Freeman, E., ... & Morris-Schaffer, K. (2024). Xylene: weight of evidence approach case study to determine the need for an extended one generation reproductive study with a developmental neurotoxicity animal cohort. *Critical Reviews in Toxicology*, *54*(10), 925-952.

Kalamkar, P., Gadgoli, C., Girase, G., Dalvi, R., Dadrekar, P., Karulkar, N., ... & Kalamkar, V. (2024). Novel biomarkers for detection of nephrotoxicity. *Journal of Renal Injury Prevention*, *13*(3), e33294-e33294.

Niaz, K., Bahadar, H., Maqbool, F., & Abdollahi, M. (2015). A review of environmental and occupational exposure to xylene and its health concerns. *EXCLI journal*, *14*, 1167.

Rad, N. K., Heydari, Z., Tamimi, A. H., Zahmatkesh, E., Shpichka, A., Barekat, M., ... & Vosough, M. (2024). Review on kidney-liver crosstalk: pathophysiology of their disorders. *Cell Journal (Yakhteh)*, *26*(2), 98.

Saggu, G., & Kumar, A. (2025). Risk assessment of Xylene and its impact on environment and human health. In *Hazardous Chemicals* (pp. 375-389). Academic Press.

Shojaee Barjoee, S., Dashtian, A. H., Keykhosravi, S. S., Abbasi Saryazdi, M. J., & Afrough, M. J. (2021). Modeling the environmental, health, and safety aspects of xylene isomer emission from storage tanks in petrochemical industries, Iran. *Environmental Monitoring and Assessment*, *193*(12), 783.

Soleimani, E. (2020). Benzene, toluene, ethylbenzene, and xylene: current analytical techniques and approaches for biological monitoring. *Reviews in Analytical Chemistry*, *39*(1), 168-187.

Suaidi, N. A., Alshawsh, M. A., Hoe, S. Z., Mokhtar, M. H., & Md Zin, S. R. (2024). Impact of xylene exposure during organogenesis on foeto-placental efficiency and foetal viability: Exploring its association with oxidative stress-induced inflammation and apoptosis in utero. *Toxicology and Industrial Health*, *40*(12), 692-710.

Suresh, A., Muninathan, N., Sampath, S., Devanesan, S., AlSalhi, M. S., & Manoj, D. (2025). Role of traditional and new biomarkers in the assessment of chronic kidney diseases: a comprehensive analysis of the biochemical, molecular and clinical dimensions. *Molecular Biology Reports*, *52*(1), 1-15.

Suresh, A., Muninathan, N., Sampath, S., Devanesan, S., AlSalhi, M. S., & Manoj, D. (2025). Role of traditional and new biomarkers in the assessment of chronic kidney diseases: a comprehensive analysis of the biochemical, molecular and clinical dimensions. *Molecular Biology Reports*, *52*(1), 1-15.

Tamber, S. S., Bansal, P., Sharma, S., Singh, R. B., & Sharma, R. (2023). Biomarkers of liver diseases. *Molecular biology reports*, *50*(9), 7815-7823.

Uboh, F. E., Ufot, S. U., Luke, U. O., & Mboso, E. (2022). Benzene, toluene, ethylene and xylene (BTEX) is implicated in gasoline-induced oxidative stress in male albino Wistar rats. *Journal of Toxicology and Environmental Health Sciences*, *14*(2), 36-41.

Verma, N., Pandit, S., Gupta, P. K., Kumar, S., Kumar, A., Giri, S. K., ... & Priya, K. (2022). Occupational health hazards and wide spectrum of genetic damage by the organic solvent fumes at the workplace: A critical appraisal. *Environmental Science and Pollution Research*, *29*(21), 30954-30966

Verzár, Z., Kiss, R., Bálint, C. P., Pakai, A., & Csákvári, T. (2025). Nutritional Management of Liver Failure in the Intensive Care Unit. *Medicina*, *61*(7), 1210.

Yameny, K. (2025). Understanding Kidney Health: Physiology, Diagnostic Markers, and Pathological Conditions. *Biobacta Journal of Biochemistry and Molecular Biology*, *2*(1), 26-38.

