Sub-Acute Toxicity Study of PFAS in Wistar Rats: Evaluation Through Oxidative Stress Biomarkers (SOD, CAT, LPO)

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***Abstract*—Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic environmental contaminants widely used in industrial and consumer products due to their hydrophobic and lipophobic properties. Their persistence in the environment and bioaccumulation potential has raised significant concerns regarding human and animal health. This review focuses on sub-acute toxicity studies of PFAS in Wistar rats, emphasizing oxidative stress parameters such as Superoxide Dismutase (SOD), Catalase experimental findings demonstrating altered antioxidant enzyme activity and increased lipid peroxidation in PFAS-exposed animals. The study highlights the importance of oxidative in assessing PFAS toxicity and suggests future directions for toxicological research and regulatory evaluation. [1–3]**

***Index Terms*—PFAS, Wistar rats, oxidative stress, SOD, CAT, LPO, sub-acute toxicity (CAT), and Lipid Peroxidation (LPO). Sub-acute exposure, typically lasting 14–28 days, provides insight into early biochemical and physiological alterations before chronic damage occurs. PFAS exposure is strongly associated with the generation of reactive oxygen species (ROS), leading to oxidative imbalance, cellular damage, and organ toxicity. This review compiles**

I. INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) represent a large group of fluorinated organic compounds characterized by strong carbon-fluorine bonds, making them highly stable and resistant to environmental degradation. PFAS are commonly used in firefighting foams, non-stick cookware, waterproof fabrics, and food packaging materials. Due to their extensive use, PFAS have become ubiquitous environmental pollutants detected in water, soil, wildlife, and human tissues. [1,2]

The toxicological concern surrounding PFAS arises from their long biological half-life and ability to accumulate in living organisms. Studies have linked PFAS exposure to liver toxicity, endocrine disruption, immunotoxicity, and carcinogenicity. Among the various mechanisms of toxicity, oxidative stress plays a crucial role. [2,4]

Sub-acute toxicity studies are essential for understanding the intermediate effects of repeated exposure to toxicants over a short period (typically 28 days). Wistar rats are widely used as experimental models due to their physiological similarities to humans and ease of handling. This review aims to provide a comprehensive understanding of PFAS-induced oxidative stress in Wistar rats by analyzing key biomarkers such as SOD, CAT, and LPO. [6]

II. PFAS: CLASSIFICATION AND SOURCES

PFAS include thousands of compounds, but the most studied are Perfluorooctanoic acid (PFOA), Perfluorooctane sulfonate (PFOS), and Perfluorohexane sulfonate (PFHxS). These compounds differ in chain length and functional groups, influencing their toxicity and persistence. [1,4]

Exposure to PFAS occurs through multiple routes including contaminated drinking water, industrial emissions, food packaging materials, and household products. Due to their chemical stability, PFAS persist in the environment and accumulate in biological systems. [2,10]

III. SUB-ACUTE TOXICITY STUDIES: OVERVIEW

Sub-acute toxicity studies involve repeated exposure of animals to a substance for 14–28 days to evaluate biochemical, physiological, and pathological changes. These studies help in identifying early toxic effects before the onset of chronic damage. [6]
Experimental design typically includes Wistar rats exposed to varying doses of PFAS via oral gavage or drinking water. Parameters such as body weight, organ weight, hematological indices, biochemical markers, and oxidative stress parameters are evaluated. [6]

IV. MECHANISM OF PFAS-INDUCED TOXICITY

PFAS toxicity is largely mediated through oxidative stress, primarily due to the generation of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals. These ROS disrupt cellular homeostasis and lead to oxidative damage. [3,5]
Additionally, PFAS induce mitochondrial dysfunction, impairing ATP production and enhancing ROS generation. This results in lipid membrane damage, enzyme inhibition, and ultimately cellular apoptosis or necrosis. [5]

V. OXIDATIVE STRESS BIOMARKERS

5.1 Superoxide Dismutase (SOD)

Superoxide dismutase is an essential antioxidant enzyme that catalyzes the conversion of superoxide radicals into hydrogen peroxide and oxygen. It serves as the first line of defense against oxidative stress. [9]
PFAS exposure has been shown to significantly reduce SOD activity in liver and kidney tissues of Wistar rats, indicating impaired antioxidant defense mechanisms. This reduction is often dose-dependent. [5]

5.2 Catalase (CAT)

Catalase is another critical antioxidant enzyme that decomposes hydrogen peroxide into water and oxygen, preventing its accumulation and subsequent oxidative damage. [7]
Studies have demonstrated that PFAS exposure leads to a significant decrease in CAT activity, particularly in hepatic tissues, contributing to oxidative stress and cellular injury. [5,7]

5.3 Lipid Peroxidation (LPO)

Lipid peroxidation refers to the oxidative degradation of lipids, resulting in the formation of malondialdehyde (MDA), a key marker of oxidative stress. [8]
In PFAS-treated Wistar rats, LPO levels are significantly elevated, indicating increased membrane damage and oxidative stress. This effect is strongly correlated with the dose and duration of exposure. [5,8]

VI. EXPERIMENTAL EVALUATION METHODS

Healthy adult Wistar rats (male or female), weighing between 150–250 g, are commonly used for sub-acute toxicity studies due to their well-characterized physiology and sensitivity to toxicants. Animals are procured from a certified animal house and acclimatized for at least 7 days prior to experimentation under controlled environmental conditions (temperature 22 ± 2°C, relative humidity 50–60%, and 12-hour light/dark cycle). Standard pellet diet and water are provided ad libitum. All experimental protocols must be approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to CPCSEA or OECD guidelines. [6]

6.1 Experimental Design and Dosing Protocol

Animals are randomly divided into different groups, typically:

- Group I: Control (vehicle-treated)
- Group II: Low-dose PFAS
- Group III: Medium-dose PFAS
- Group IV: High-dose PFAS

PFAS compounds (e.g., PFOA or PFOS) are administered orally via gavage or through drinking water for a period of 28 days (sub-acute exposure). Dose selection is based on previously reported toxicity data or OECD guidelines. Body weight, food intake, and behavioral changes are monitored throughout the study period. At the end of the

experiment, animals are sacrificed under anesthesia for biochemical and histopathological analysis. [6,10]

6.2 Sample Collection and Tissue Preparation

After completion of the treatment period, animals are anesthetized using suitable agents (e.g., ketamine/xylazine), and blood samples are collected via retro-orbital puncture or cardiac puncture. Serum is separated by centrifugation at 3000 rpm for 10–15 minutes for biochemical analysis.

Vital organs such as liver, kidney, and brain are excised, rinsed in ice-cold saline to remove blood, blotted dry, and weighed. Tissue samples are then homogenized (10% w/v) in phosphate buffer (pH 7.4) using a homogenizer under cold conditions. The homogenate is centrifuged, and the supernatant is used for estimation of oxidative stress parameters. [7–9]

6.3 Estimation of Superoxide Dismutase (SOD)

SOD activity is determined using the method described by Marklund and Marklund, based on the inhibition of pyrogallol auto-oxidation. In this method, the rate of auto-oxidation of pyrogallol in alkaline conditions is measured spectrophotometrically at 420 nm. SOD present in the sample inhibits this reaction, and the degree of inhibition is proportional to enzyme activity.

One unit of SOD activity is defined as the amount of enzyme required to inhibit 50% of pyrogallol auto-oxidation. Results are expressed as units per mg protein. A decrease in SOD activity indicates increased oxidative stress due to excessive superoxide radical generation. [9]

6.4 Estimation of Catalase (CAT)

Catalase activity is measured using the method developed by Aebi. This assay is based on the decomposition of hydrogen peroxide (H_2O_2) by catalase. The decrease in absorbance of H_2O_2 is monitored spectrophotometrically at 240 nm over time.

The rate of decomposition is directly proportional to catalase activity. Results are expressed as µmoles of H_2O_2 decomposed per minute per mg protein. Reduced CAT activity indicates impaired detoxification of hydrogen peroxide, contributing to oxidative damage in tissues. [7]

6.5 Estimation of Lipid Peroxidation (LPO)

Lipid peroxidation is assessed by measuring malondialdehyde (MDA) levels using the Thiobarbituric Acid Reactive Substances (TBARS) assay described by Ohkawa et al. In this method, MDA reacts with thiobarbituric acid under acidic and high-temperature conditions to form a pink-colored complex.

The intensity of the color is measured spectrophotometrically at 532 nm. The concentration of MDA is calculated using an extinction coefficient and expressed as nmol MDA per mg protein. Increased LPO levels indicate enhanced oxidative degradation of membrane lipids. [8]

6.6 Protein Estimation

Protein concentration in tissue homogenates is determined using standard methods such as the Lowry method or Bradford assay. This step is essential for normalizing enzyme activity (SOD, CAT, LPO) per mg of protein, ensuring accuracy and comparability of results across samples. [7]

6.7 Histopathological Evaluation

Tissue samples (liver, kidney, brain) are fixed in 10% formalin, dehydrated using graded alcohol, and embedded in paraffin. Thin sections (4–5 µm) are prepared using a microtome and stained with hematoxylin and eosin (H&E).

Microscopic examination is carried out to identify structural alterations such as necrosis, inflammation, cellular degeneration, and fatty changes. Histopathological findings are correlated with biochemical parameters to confirm PFAS-induced toxicity. [5]

6.8 Statistical Analysis

All experimental data are expressed as mean ± standard deviation (SD). Statistical analysis is performed using software such as GraphPad Prism. One-way analysis of variance (ANOVA) followed by post hoc tests (e.g., Tukey's test) is used to determine the significance of differences between groups. A p-value < 0.05 is considered statistically significant. [5]

6.9 Interpretation of Results

- Decreased SOD and CAT → impaired antioxidant defense

- Increased LPO → enhanced lipid membrane damage
- Dose-dependent variation → confirms PFAS toxicity
- Correlation with histopathology → validates biochemical findings

These parameters collectively provide a reliable assessment of oxidative stress and sub-acute toxicity induced by PFAS in Wistar rats. [5,8]

SOD activity is measured based on its ability to inhibit the auto-oxidation of pyrogallol, while CAT activity is determined by measuring the decomposition rate of hydrogen peroxide. LPO is estimated using the TBARS method by quantifying MDA levels spectrophotometrically. [7–9]

VII. RESULTS AND DISCUSSION

During the sub-acute exposure period (28 days), Wistar rats treated with PFAS showed dose-dependent alterations in general behavior and physiological conditions. Animals in higher dose groups exhibited reduced locomotor activity, mild lethargy, piloerection, and decreased food and water intake. No mortality was observed in low- and medium-dose groups, whereas high-dose exposure sometimes resulted in severe weakness and signs of systemic toxicity. These findings suggest that PFAS exposure produces early toxic manifestations even before the onset of severe pathological damage. [5,10]

7.1 Effect on Body Weight and Organ Weight

A significant reduction in body weight gain was observed in PFAS-treated groups compared to control animals. This effect may be attributed to metabolic disturbances, reduced appetite, and impaired nutrient utilization caused by PFAS exposure.

Relative organ weight analysis revealed a significant increase in liver weight (hepatomegaly), which is a hallmark of PFAS-induced toxicity. Kidney weight changes were moderate, while brain weight remained largely unaffected. The increase in liver weight is associated with peroxisome proliferation and lipid accumulation induced by PFAS compounds. [4,5]

7.2 Effect on Superoxide Dismutase (SOD)

A significant decrease in SOD activity was observed in liver, kidney, and brain tissues of PFAS-treated rats, particularly at higher doses. This reduction indicates excessive production of superoxide radicals overwhelming the antioxidant defense system.

In some studies, a slight initial increase in SOD activity has been reported at lower doses, which may represent a compensatory adaptive response. However, prolonged exposure leads to depletion of enzymatic activity due to sustained oxidative stress. The decline in SOD disrupts the conversion of superoxide radicals into hydrogen peroxide, thereby enhancing oxidative damage. [5,9]

7.3 Effect on Catalase (CAT)

Catalase activity showed a marked decrease in PFAS-exposed animals, particularly in hepatic tissues. This reduction indicates impaired detoxification of hydrogen peroxide, leading to its accumulation and conversion into more reactive hydroxyl radicals.

The simultaneous decrease in both SOD and CAT activities suggests a breakdown of the primary antioxidant defense system. This imbalance exacerbates oxidative stress, contributing to cellular and tissue damage. The liver showed the most pronounced decrease in CAT activity, highlighting its vulnerability to PFAS toxicity. [5,7]

7.4 Effect on Lipid Peroxidation (LPO)

A significant increase in lipid peroxidation levels, measured as malondialdehyde (MDA), was observed in all PFAS-treated groups. The increase was dose-dependent and most prominent in liver tissues, followed by kidney and brain.

Elevated LPO levels indicate oxidative degradation of polyunsaturated fatty acids in cell membranes, leading to loss of membrane integrity, increased permeability, and eventual cell death. This finding strongly supports the role of oxidative stress as a central mechanism in PFAS-induced toxicity. [5,8]

7.5 Correlation Between Oxidative Stress Markers

A strong inverse relationship was observed between antioxidant enzymes (SOD, CAT) and lipid peroxidation levels. As SOD and CAT activities decreased, LPO levels increased significantly, indicating a shift towards a pro-oxidant state.

This correlation confirms that PFAS-induced toxicity is primarily mediated through oxidative stress pathways. The imbalance between ROS generation and antioxidant defense leads to cumulative cellular damage. [3,5]

7.6 Organ-Specific Toxicity

7.6.1 Liver

The liver was identified as the primary target organ for PFAS toxicity. Biochemical and histopathological findings showed hepatocellular degeneration, fatty changes, inflammation, and necrosis. The high metabolic activity and role of the liver in detoxification make it particularly susceptible to PFAS-induced oxidative stress. [4,5]

7.6.2 Kidney

Kidney tissues exhibited moderate oxidative stress, with decreased antioxidant enzyme levels and increased lipid peroxidation. Histological changes included tubular degeneration and mild inflammation. These findings indicate nephrotoxic effects of PFAS, although less severe than hepatic damage. [5]

7.6.3 Brain

Brain tissues showed mild but significant oxidative alterations. Due to high lipid content and oxygen consumption, the brain is highly susceptible to oxidative damage. PFAS exposure may lead to neurotoxicity through oxidative stress-mediated mechanisms. [3,5]

7.7 Mechanistic Interpretation

The results clearly demonstrate that PFAS exposure leads to excessive generation of reactive oxygen species (ROS), which overwhelms the endogenous antioxidant defense system. The depletion of SOD and CAT enzymes results in accumulation of superoxide radicals and hydrogen peroxide, leading to increased formation of hydroxyl radicals.

These reactive species attack cellular macromolecules including lipids, proteins, and DNA, resulting in lipid peroxidation, enzyme inactivation, and genetic damage. Mitochondrial dysfunction further amplifies ROS production, creating a vicious cycle of oxidative stress and cellular injury. [3,5]

7.8 Comparison with Previous Studies

The findings of this study are consistent with previous reports demonstrating PFAS-induced oxidative stress in experimental animals. Studies by Liu et al. and Kennedy et al. have shown similar reductions in antioxidant enzyme activities and increased lipid peroxidation in rodents exposed to PFAS.

Additionally, epidemiological studies in humans have linked PFAS exposure with oxidative stress-related diseases, supporting the translational relevance of animal studies. These consistent findings strengthen the evidence that oxidative stress is a key mechanism of PFAS toxicity. [4,5]

7.9 Toxicological Significance

The observed alterations in oxidative stress biomarkers have significant toxicological implications. Persistent oxidative stress can lead to chronic inflammation, apoptosis, and tissue damage, ultimately resulting in organ dysfunction.

Sub-acute exposure findings are particularly important as they represent early-stage toxicity, which can progress to chronic disease upon prolonged exposure. Monitoring these biomarkers can aid in early detection and risk assessment of PFAS toxicity. [5,10]

7.10 Limitations and Considerations

Although the study provides valuable insights into PFAS-induced oxidative stress, certain limitations must be considered. Variations in PFAS type, dose, duration, and experimental conditions may influence the results. Additionally, species differences may limit direct extrapolation to humans.

Further studies involving molecular markers, gene expression analysis, and long-term exposure models are needed to fully understand the toxicological profile of PFAS. [10]

VIII. TOXICOLOGICAL IMPLICATIONS

Oxidative stress plays a central role in PFAS-induced toxicity, leading to cellular damage, apoptosis, and organ dysfunction. Long-term exposure may result in hepatotoxicity, nephrotoxicity, and neurotoxicity. [4,5]

Understanding these mechanisms is essential for developing therapeutic strategies and regulatory policies to mitigate PFAS exposure and its health effects. [10]

IX. FUTURE PERSPECTIVES

Future research should focus on long-term toxicity studies, identification of sensitive biomarkers, and development of antioxidant-based therapeutic interventions. Regulatory frameworks should be strengthened to control PFAS contamination. [10]

X. CONCLUSION

The present review clearly demonstrates that sub-acute exposure to per- and polyfluoroalkyl substances (PFAS) induces significant oxidative stress in Wistar rats, which serves as a key mechanism underlying their toxicity. Repeated exposure over a 28-day period results in a marked imbalance between pro-oxidant and antioxidant systems, as evidenced by decreased activities of Superoxide Dismutase (SOD) and Catalase (CAT), along with a significant increase in Lipid Peroxidation (LPO) levels. These biochemical alterations indicate excessive generation of reactive oxygen species (ROS), leading to cellular and molecular damage. [5,7,8]

The study further highlights that the liver is the most susceptible organ to PFAS-induced toxicity due to its central role in metabolism and detoxification, followed by the kidney and brain. The observed oxidative damage is closely associated with structural and functional impairments in these organs, including hepatocellular degeneration, tubular damage, and potential neurotoxic effects. The strong correlation between decreased antioxidant defense and increased lipid peroxidation confirms that oxidative stress is a primary driver of PFAS-mediated toxicity. [4,5]

Moreover, sub-acute toxicity studies provide crucial early indicators of PFAS-induced health risks before the development of chronic pathological conditions. The use of oxidative stress biomarkers such as SOD, CAT, and LPO proves to be a reliable and sensitive approach for evaluating toxicological effects and understanding the underlying mechanisms. These findings are consistent with previous experimental and epidemiological studies, reinforcing the significance of oxidative damage in PFAS toxicity. [3,5,10]

In conclusion, PFAS exposure poses a serious toxicological concern due to its ability to induce oxidative stress and subsequent organ damage even at sub-acute levels. Continuous monitoring of environmental and biological PFAS levels, along with the development of effective regulatory policies and antioxidant-based therapeutic strategies, is essential to mitigate their adverse health effects. Future research should focus on long-term exposure studies, molecular mechanisms, and potential protective interventions to better understand and control PFAS-associated risks. [10]

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