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ORIGINAL RESEARCH ARTICLE

Chrysin attenuates pentadecafluorooctanoic acid-induced hepatotoxicity in male Wistar rats via modulation of oxidative stress, inflammation, and metabolic dysfunction

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Abstract

Pentadecafluorooctanoic acid (PFOA), a persistent environmental pollutant, has been implicated in hepatotoxicity through mechanisms involving oxidative stress, inflammation, and metabolic dysfunction. This study investigated the hepatoprotective effects of chrysin against PFOA-induced liver injury in male Wistar rats. Twenty-five male Wistar rats were randomly divided into five groups (n = 5): control (20% dimethyl sulfoxide), PFOA (5 mg/kg), PFOA + chrysin (25 mg/kg), PFOA + chrysin (50 mg/kg), and chrysin only (50 mg/kg). Treatments were orally administered for 14 days. Serum and hepatic biomarkers of liver function, oxidative stress, inflammation, membrane integrity, and energy metabolism were evaluated using standard biochemical assays and enzyme-linked immunosorbent assay methods. PFOA exposure significantly ($p < 0.05$) increased serum liver enzymes (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and lactate dehydrogenase), lipid peroxidation marker (malondialdehyde), and pro-inflammatory cytokines (interleukin 1 beta and interferon gamma), while significantly reducing antioxidant parameters (glutathione, catalase, superoxide dismutase, glutathione peroxidase, and glutathione-S-transferase, nitric oxide (NO), and membrane-bound enzyme activities, including adenosine triphosphatase (ATPase), Ca^{2+}/Mg^{2+} ATPase, and 5′-nucleotidase. Chrysin treatment at both 25 and 50 mg/kg significantly ($p < 0.05$) ameliorated these alterations in a dose-dependent manner, with the 50 mg/kg dose showing greater protective efficacy. The findings indicate that PFOA-induced hepatotoxicity is associated with oxidative stress, inflammation, and metabolic dysfunction, whereas chrysin pre-treatment restores antioxidant defenses, attenuates inflammatory responses, and normalizes membrane-associated enzyme activities. Overall, chrysin demonstrates potent protective potential against PFOA-induced liver injury and may serve as a promising therapeutic candidate for environmental toxin-associated hepatotoxicity.

Keywords: Chrysin; Pentadecafluorooctanoic acid; Hepatotoxicity; Oxidative stress; Inflammation; Antioxidant defense

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1. Introduction

The liver is a vital organ central to regulating numerous physiological processes, including metabolism, detoxification, protein synthesis, and nutrient storage.[1,2] Its role in detoxifying harmful substances makes it particularly vulnerable to xenobiotics, substances foreign to biological systems, which can cause significant hepatic injury.[3] Among these xenobiotics, pentadecafluorooctanoic acid (PFOA), a per- and polyfluoroalkyl substance commonly referred to as a "forever chemical," widely used in industrial and consumer products due to its water- and oil-resistant properties, stands out as a persistent environmental pollutant.[4] PFOA's non-biodegradable nature has led to environmental accumulation and bioaccumulation in living organisms, where it exerts toxic effects, particularly on the liver.[5] Hepatotoxicity, referring to liver damage caused by chemical substances, remains a significant public health concern.[6] PFOA has raised alarm due to its persistence in the environment and adverse biological effects, including disruptions in lipid metabolism, increased oxidative stress, and elevated liver enzyme levels, contributing to hepatocellular damage.[7–10] Its stable carbon–fluorine bonds make it resistant to degradation, causing it to accumulate in biological systems, including humans and animals.[11,12]

Exposure to PFOA induces hepatotoxicity, which is typically manifested by increased serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), established biochemical indicators of hepatocellular injury.[8,10,13,14] Lactate dehydrogenase (LDH) reflects cellular membrane integrity and anaerobic metabolic shifts during oxidative stress.[14] At the cellular level, PFOA increases lipid peroxidation (malondialdehyde [MDA]) while depleting antioxidants like glutathione (GSH) and impairing the activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione-S-transferase (GST).[9,15–17] GST, a phase II detoxification enzyme, plays a critical role in conjugating reactive intermediates but has been overlooked in prior PFOA studies. Concurrently, PFOA disrupts hepatic metabolism by suppressing 5'-nucleotidase (5'-NT) and adenosine triphosphatase (ATPase) activities.[18,19] The former is a sensitive indicator of cholestasis, while the latter, particularly Ca^{2+}/Mg^{2+} ATPase, is essential for ion homeostasis and bile secretion.[20,21] Nitric oxide (NO) depletion further exacerbates injury by impairing vasodilation and anti-inflammatory signaling.[22] The inflammatory cascade triggered by PFOA involves upregulation of pro-inflammatory cytokines (interleukin [IL]-1β, interferon gamma [IFN-γ]) and myeloperoxidase (MPO), a marker of neutrophil infiltration and oxidative burst.[23,24]

Natural compounds like chrysin, a flavonoid naturally present in honey, passion flowers, and propolis, have drawn considerable attention for their hepatoprotective properties.[25,26] Chrysin is widely recognized for its antioxidant, anti-inflammatory, and hepatoprotective effects, making it a promising therapeutic candidate for managing liver injuries.[27,28] Its potent ability to scavenge reactive oxygen species (ROS) and enhance endogenous antioxidant defenses helps counteract oxidative stress, a major driver of liver damage.[29–32] In the liver, excessive ROS generation can result in lipid peroxidation, protein damage, and enzyme inactivation, which, if unaddressed, culminates in hepatocellular injury.[33,34] Chrysin's antioxidant properties mitigate these effects by neutralizing free radicals and boosting the activity of endogenous antioxidant enzymes, such as GPx, SOD, and CAT, thereby protecting liver cells from oxidative damage.[35,36] In addition to its antioxidant capacity, chrysin suppresses pro-inflammatory mediators such as IL-1β and IFN-γ, further reinforcing its potential in mitigating liver inflammation and toxicity, particularly those caused by PFOA exposure.[28]

Despite growing evidence that PFOA disrupts hepatic homeostasis through oxidative stress and inflammation, the biochemical mechanisms underlying its hepatotoxicity remain incompletely characterized, particularly with respect to inflammatory mediators, antioxidant defenses, membrane integrity, and energy metabolism. Moreover, limited information is available regarding the ability of natural flavonoids such as chrysin to simultaneously modulate these interconnected processes during PFOA-induced liver injury. Although chrysin has demonstrated antioxidant and hepatoprotective activities in several experimental models, its efficacy against PFOA-induced hepatotoxicity has not been comprehensively investigated. To the best of our knowledge, this study is among the first to evaluate the hepatoprotective potential of chrysin against PFOA-induced liver injury through an integrated assessment of hepatic function, oxidative stress, inflammatory responses, membrane integrity, and metabolic dysfunction. Therefore, the aim of this study is to investigate whether chrysin pre-treatment attenuates PFOA-induced hepatotoxicity in male Wistar rats by restoring hepatic antioxidant defenses, reducing inflammatory responses, preserving membrane integrity, and improving metabolic homeostasis.

2. Materials and methods

2.1. Chemicals and reagents

Pentadecafluorooctanoic acid, chrysin, and dimethyl sulfoxide were obtained from Sigma-Aldrich (United

States). ALT (cat no. AL 1200), AST (cat no. AS 1202), ALP (cat no. AP 542), and total protein assay kits were purchased from Randox Laboratories Ltd. (United Kingdom). Enzyme-linked immunosorbent assay (ELISA) kits for IFN-γ (cat no. E-EL-R0009) and IL-1β cat no. E-EL-R0012) were procured from ElabScience (United States). The Griess reagent kit used for NO determination was obtained from Cayman Chemical (United States).

Other analytical reagents used for biochemical assays, including, reduced nicotinamide adenine dinucleotide, hydrogen peroxide, thiobarbituric acid, ascorbic acid, trichloroacetic acid, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, magnesium chloride, calcium chloride, ATP, reduced GSH, oxidized glutathione, 1-chloro-2,4-dinitrobenzene, Ellman's reagent [5,5'-dithiobis(2-nitrobenzoic acid)], pyrogallol, hydrazine sulfate, and ammonium molybdate were purchased from Sigma-Aldrich (United States). Sodium dodecyl sulfate was obtained from British Drug Houses (United Kingdom). All reagents used in this study were of analytical grade.

2.2. Experimental animals

A total of 25 male Wistar rats aged 6 – 8 weeks and weighing between 120 and 180 g were obtained from the animal facility of the Department of Biochemistry, College of Biosciences, Federal University of Agriculture, Abeokuta, Nigeria. Male rats were selected to avoid hormonal fluctuations associated with the estrous cycle in females, which may influence metabolic, oxidative stress, and biochemical parameters.[37,38] The animals were housed in well-ventilated cages under standard laboratory conditions (temperature: 22 °C ± 2 °C, relative humidity: 50%–60%, and a 12-h light/dark cycle). Rats were acclimatized for 14 days prior to the commencement of the experiment and were provided with standard laboratory chow and water *ad libitum*.[39]

All experimental procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals. Humane care and use of animals in this study were conducted in accordance with the Animal Research: Reporting of *In Vivo* Experiments guidelines 2.0.[40] Ethical approval was obtained from the Animal Ethics Committee of the Federal University of Agriculture, Abeokuta (Approval No. FUNAAB/COLBIOS/BCH/UG/20201184).

2.3. Experimental design

The animals were randomly assigned to five experimental groups consisting of five rats per group. The treatment protocol lasted for 14 consecutive days. Prior to administration, PFOA and chrysin were dissolved in 20% dimethyl sulfoxide to obtain the desired working concentrations.[41–45] Hepatotoxicity was induced by oral administration of PFOA once daily. Chrysin was also administered prior to PFOA exposure to evaluate its potential protective effect against PFOA-induced hepatotoxicity. The experimental design and treatment regimen followed the protocol previously established by Awolesi *et al.*[39] The treatment groups were as follows:

(i) Group 1 (Control): Rats received 20% dimethyl sulfoxide only.
(ii) Group 2 (PFOA): Rats received PFOA (5 mg/kg body weight).
(iii) Group 3 (Chrysin 25 mg/kg + PFOA): Rats were pre-treated with chrysin (25 mg/kg body weight), followed by PFOA (5 mg/kg body weight) two h later.
(iv) Group 4 (Chrysin 50 mg/kg + PFOA): Rats were pre-treated with chrysin (50 mg/kg body weight), followed by PFOA (5 mg/kg body weight) two h later.
(v) Group 5 (Chrysin only): Rats received chrysin (50 mg/kg body weight) alone.

2.4. Sample collection and preparation

At the end of the 14-day treatment period, the animals were fasted overnight prior to sample collection. The rats were anesthetized using ketamine to minimize stress during the procedure. Blood samples were collected via ocular puncture and allowed to clot, after which serum was obtained by centrifugation at 3,500 rpm for 10 min. Following blood collection, the animals were humanely euthanized by cervical dislocation, and the liver tissues were immediately excised, rinsed in ice-cold saline to remove blood residues, blotted dry, and weighed.[46]

2.5. Biochemical assays and analysis

For biochemical analysis, liver tissues were homogenized to obtain a 10% (w/v) homogenate using ice-cold 0.1 M phosphate-buffered saline (pH 7.4).[47] The homogenates were centrifuged at 4,000×g for 10 min at 4 °C, and the resulting supernatants were collected for biochemical assays. Both serum and tissue supernatants were aliquoted and stored at −80 °C until further analysis.[39,48]

2.5.1. Determination of protein concentration

Total protein concentrations in serum and liver homogenates were determined spectrophotometrically using the method of Lowry *et al.*[49] with bovine serum albumin as the standard. The assay was performed according to the protocol provided with the Randox total protein assay kit (Randox Laboratories, Northern Ireland). Enzyme activities were normalized to protein content to account for variations in tissue concentration.

2.5.2. Liver enzyme activities

Serum and hepatic ALT and AST activities were determined using Randox diagnostic kits based on the method described by Reitman and Frankel.[50] ALP activity was measured using the method of Akuyam *et al.*[51], which involves the hydrolysis of p-nitrophenyl phosphate to p-nitrophenol. LDH activity was assayed according to the procedure described by Holmes & Scopes[52] as modified by Al-Jassabi[53], based on the oxidation of reduced nicotinamide adenine dinucleotide during the conversion of pyruvate to lactate.

2.5.3. Oxidative stress markers

Lipid peroxidation was assessed by measuring MDA levels using the thiobarbituric acid reactive substances assay as described by Jabba.[54] NO levels were determined using the Griess reaction method described by Green *et al.*[55] as modified by Hodges *et al.*[56], which quantifies nitrite levels as an indicator of nitric oxide production.

2.5.4. Enzymatic antioxidants

Glutathione peroxidase activity was determined according to the method of Rotruck *et al.*[57], which measures the conversion of reduced GSH to oxidized glutathione in the presence of hydrogen peroxide as described by Akamo *et al.*[58] CAT activity was assessed based on the decomposition of hydrogen peroxide as described by Hadwan and Abed.[59] SOD activity was determined by measuring the inhibition of pyrogallol autoxidation according to Sun *et al.*[60] GST activity was measured using 1-chloro-2,4-dinitrobenzene as the substrate following the method of Tew and Ronai.[61]

2.5.5. Non-enzymatic antioxidants

Reduced GSH levels were determined using Ellman's reagent, which reacts with thiol groups to form a yellow chromogen according to the method described by Ellman.[62] Total antioxidant capacity was evaluated using the phosphomolybdenum assay as described by Prieto *et al.*[63], which quantifies the reduction of Mo(VI) to Mo(V).

2.5.6. Membrane-bound enzyme activities

Total ATPase activity was measured by determining the amount of inorganic phosphate released during ATP hydrolysis according to Tsakiris and Deliconstantinos.[64] Ca^{2+}/Mg^{2+} ATPase activity was determined in the presence of calcium and magnesium ions following the method of Hanahan and Ekholm.[65] 5'-NT activity was quantified spectrophotometrically based on its ability to hydrolyze adenosine monophosphate according to Heinz *et al.*[66]

2.5.7. Inflammatory markers

Myeloperoxidase activity was determined using the guaiacol oxidation method in the presence of hydrogen peroxide as described by Hasmann *et al.*[67], with absorbance measured at 470 nm. Levels of the pro-inflammatory cytokines IL-1β and IFN-γ were determined using commercially available ELISA kits obtained from ElabScience (United States), following the manufacturer's instructions.

2.6. Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance followed by Tukey's post hoc test to determine significant differences between groups. Statistical significance was considered at $p < 0.05$. All analyses were performed using Statistical Package for Social Science (version 23.0), and graphs were plotted using GraphPad Prism (version 10.0).

3. Results

3.1. Effect of chrysin pre-treatment on liver enzymes in pentadecafluorooctanoic acid-induced hepatotoxicity in male Wistar rats

Pentadecafluorooctanoic acid-induced hepatotoxicity significantly increased ($p < 0.05$) serum ALT, AST, and ALP activities while reducing their levels in the liver (Figure 1). Chrysin pre-treatment (25 and 50 mg/kg) significantly ($p < 0.05$) reversed these effects, restoring serum and liver enzyme activities to normal levels. LDH activity, elevated by PFOA in both serum and liver, was also reduced by chrysin ($p < 0.05$).

3.2. Effect of chrysin on oxidative stress markers in pentadecafluorooctanoic acid-induced hepatotoxicity in male Wistar rats

Pentadecafluorooctanoic acid exposure significantly increased MDA levels ($p < 0.05$) and reduced GSH and total antioxidant capacity ($p < 0.05$) levels. Chrysin pre-treatment (25 and 50 mg/kg) effectively reversed these effects, significantly reducing MDA elevation ($p < 0.05$), restoring GSH levels ($p < 0.05$), and improving total antioxidant capacity ($p < 0.05$) compared to the PFOA-only group (Figure 2).

3.3. Effect of chrysin on antioxidant enzymes in pentadecafluorooctanoic acid-induced hepatotoxicity in the liver of male Wistar rats

Pentadecafluorooctanoic acid exposure significantly decreased ($p < 0.05$) hepatic CAT, SOD, GPx, and GST activities compared to controls. Chrysin pre-treatment (25 and 50 mg/kg) effectively reversed these effects, significantly restoring ($p < 0.05$) all enzyme activities (Figure 3).

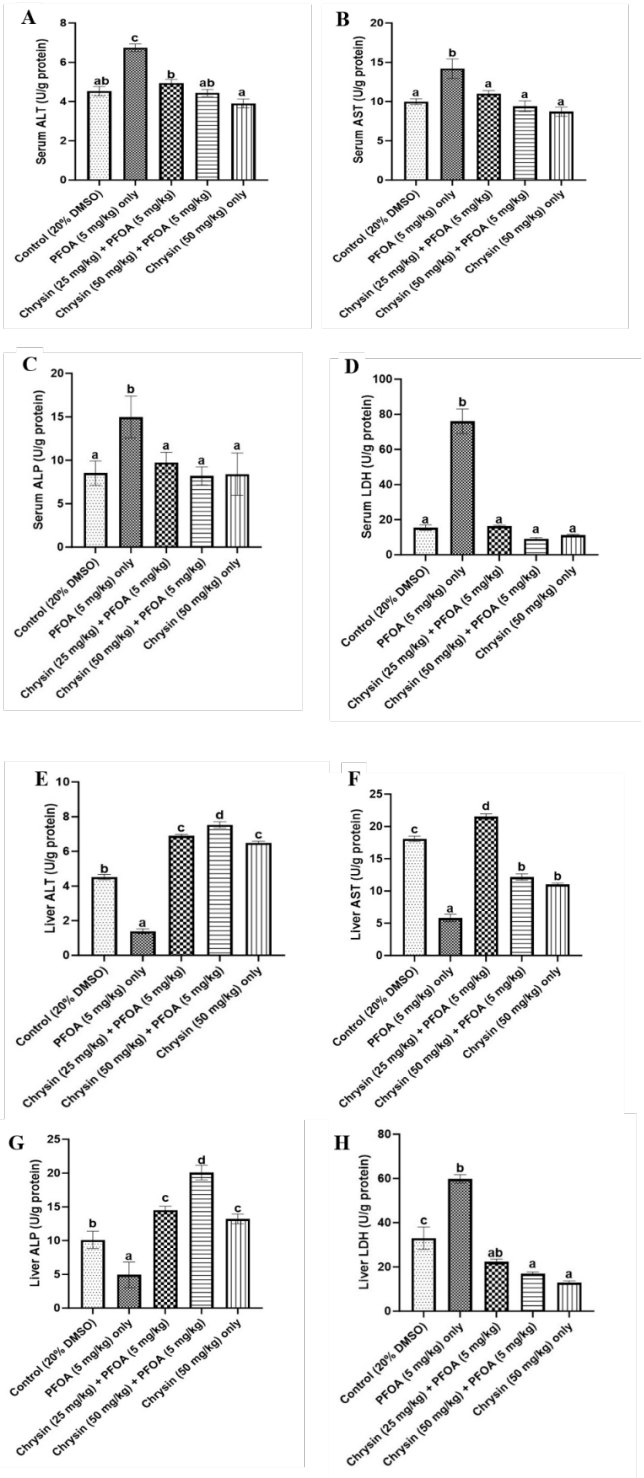


Figure 1. Effect of chrysin pre-treatment on liver enzymes in pentadecafluorooctanoic acid (PFOA)-induced hepatotoxicity in male Wistar rats. Serum (A) ALT, (B) AST, (C) ALP, and (D) LDH. Liver (E) ALT, (F) AST, (G) ALP, and (H) LDH. Data were analyzed using analysis of variance and expressed as mean ± SEM (n = 5). Bars with different letters are statistically distinct at $p < 0.05$.

Abbreviations: ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; LDH: Lactate dehydrogenase; SEM: Standard error of the mean.

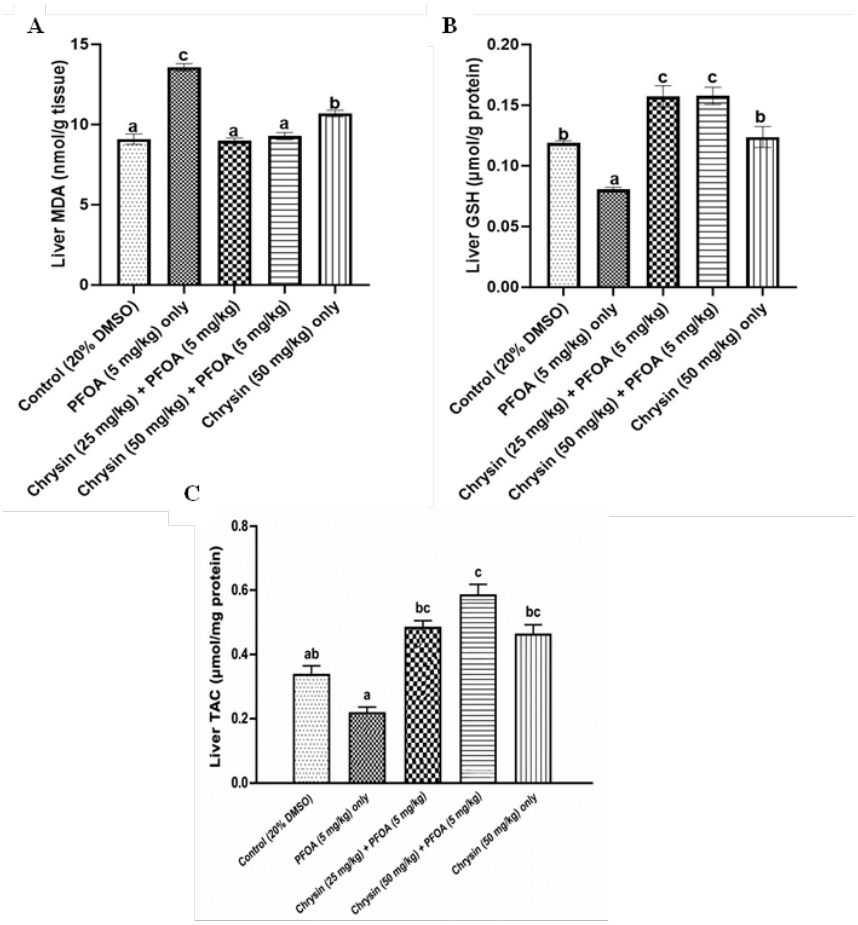


Figure 2. Effect of chrysin pre-treatment on oxidative stress markers in pentadecafluorooctanoic acid (PFOA)-induced hepatotoxicity in male Wistar rats. (A) Liver MDA, (B) liver GSH, and (C) liver TAC. Data are expressed as mean ± SEM ($n = 5$). Bars with different letters are statistically distinct at $p < 0.05$. Abbreviations: GSH: Reduced glutathione; MDA: Malondialdehyde; SEM: Standard error of the mean; TAC: Total antioxidant capacity.

3.4. Effect of chrysin on nitric oxide levels, 5'-Nucleotidase, and myeloperoxidase activities in pentadecafluorooctanoic acid-induced hepatotoxicity in male Wistar rats

Pentadecafluorooctanoic acid exposure significantly lowered NO and 5'-NT activity while increasing MPO levels ($p < 0.05$). Chrysin pre-treatment (25 and 50 mg/kg) reversed these effects, restoring NO and 5'-NT activity and reducing MPO activity in both serum and liver ($p < 0.05$) (Figure 4).

3.5. Effect of chrysin on membrane-bound enzyme activities in pentadecafluorooctanoic acid-induced hepatotoxicity in male Wistar rats

Administration of PFOA resulted in a significant decrease ($p < 0.05$) in the activities of membrane-bound enzymes compared to the control group. However, pre-treatment with chrysin at doses of 25 and 50 mg/kg body weight significantly increased ($p < 0.05$) the activities of both total ATPase and Ca^{2+}/Mg^{2+} ATPase compared to the PFOA-only group (Figure 5).

3.6. Effect of chrysin on pro-inflammatory cytokine levels in pentadecafluorooctanoic acid-induced hepatotoxicity in male Wistar rats

Pentadecafluorooctanoic acid exposure significantly increased ($p < 0.05$) both IL-1β and IFN-γ levels in the serum. Chrysin pre-treatment (25 and 50 mg/kg) effectively countered these effects, significantly reducing ($p < 0.05$) both pro-inflammatory cytokines (Figure 6).

4. Discussion

The findings of this study provide significant insight into the hepatotoxic effects of PFOA and the multifaceted protective properties of the natural flavonoid chrysin.

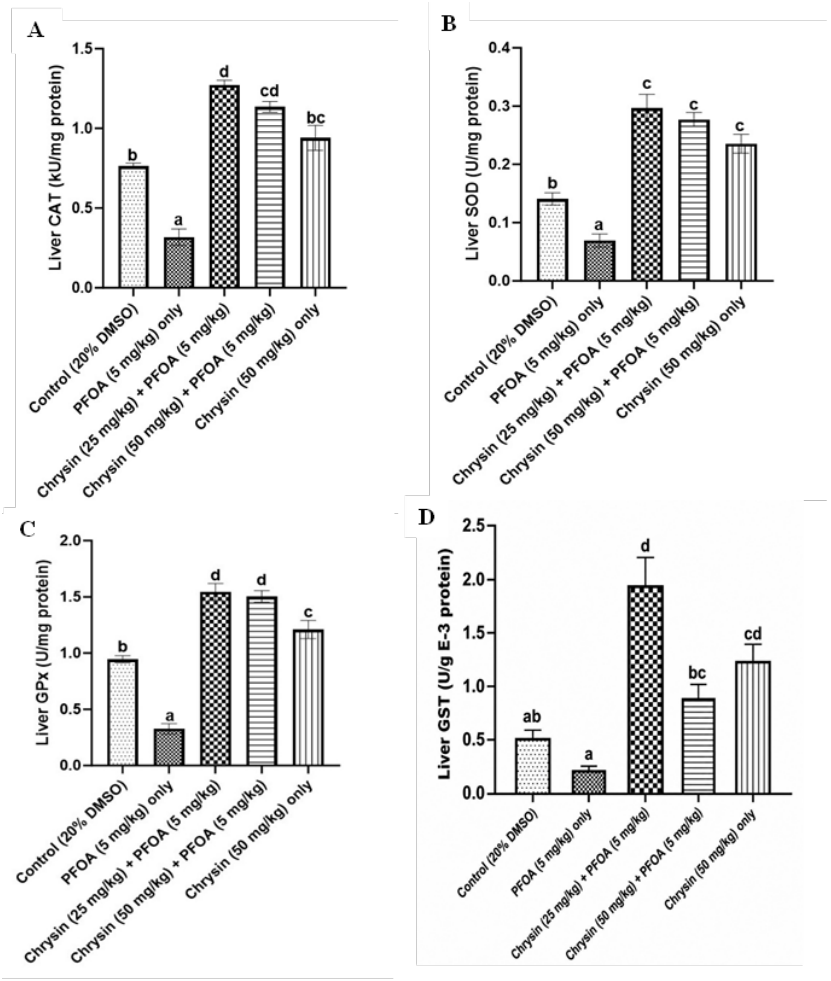


Figure 3. Effect of chrysin pre-treatment on antioxidant enzymes in pentadecafluorooctanoic acid (PFOA)-induced hepatotoxicity in male Wistar rats. (A) Liver CAT, (B) liver SOD, (C) liver GPx, and (D) liver GST. Data are expressed as mean ± SEM (n = 5). Bars with different letters are statistically distinct at $p < 0.05$.

Abbreviations: CAT: Catalase; GPx: Glutathione peroxidase; GST: Glutathione S-transferase; SEM: Standard error of the mean; SOD: Superoxide dismutase.

PFOA, a member of the per- and polyfluoroalkyl substances family, disrupts multiple hepatic metabolic pathways, resulting in oxidative stress, inflammatory responses, mitochondrial dysfunction, and metabolic disturbances that ultimately impair liver function.[68]

Chrysin (5,7-dihydroxyflavone) is a naturally occurring flavonoid widely distributed in honey, propolis, passion flowers (*Passiflora* species), and several medicinal plants. It has attracted considerable attention because of its potent antioxidant, anti-inflammatory, anti-apoptotic, and cytoprotective properties, which have demonstrated therapeutic potential in various experimental models of hepatic injury.[69] Pharmacodynamically, chrysin exerts its protective effects through free radical scavenging, enhancement of endogenous antioxidant defenses, modulation of inflammatory mediators, preservation of mitochondrial function, and regulation of apoptosis-related signaling pathways. Pharmacokinetic studies, however, have shown that despite its broad biological activities, chrysin exhibits poor oral bioavailability owing to limited aqueous solubility, extensive intestinal metabolism, and rapid glucuronidation and sulfation, which substantially reduce its systemic exposure following oral administration.[69–72] Nevertheless, experimental studies have consistently demonstrated that chrysin ameliorates chemically induced liver injury by restoring endogenous antioxidant defenses, reducing lipid peroxidation, suppressing inflammatory cytokines, and preserving hepatocellular integrity.[32] Consistent with these findings, pre-treatment with chrysin at doses of 25 and 50 mg/kg body weight produced significant ($p < 0.05$)

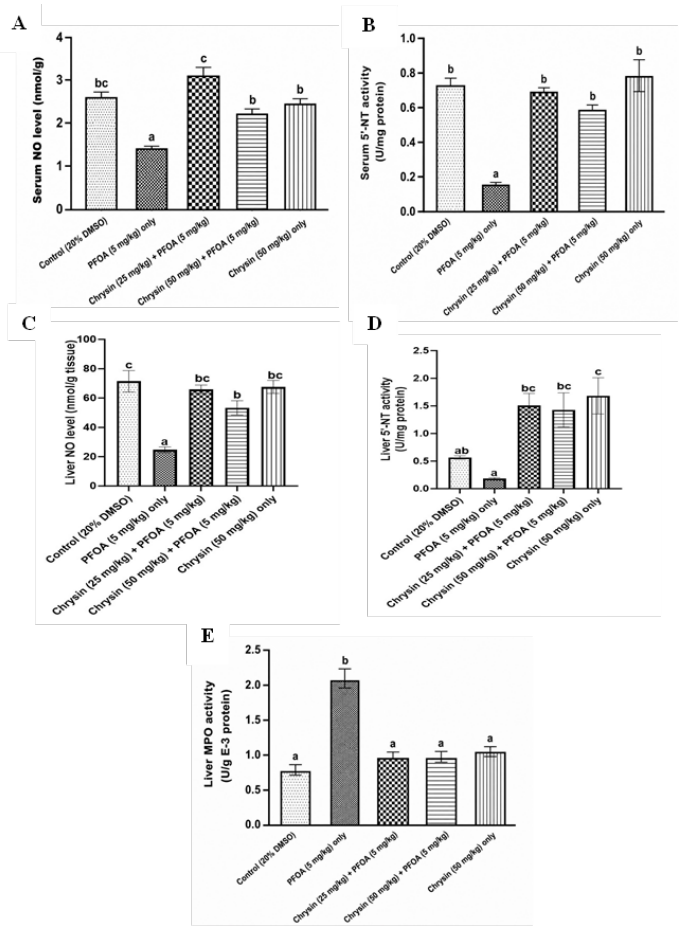


Figure 4. Effect of chrysin pre-treatment on nitric oxide (NO) levels, 5'-nucleotidase (5'-NT), and myeloperoxidase (MPO) activities in pentadecafluorooctanoic acid (PFOA)-induced hepatotoxicity in male Wistar rats. (A) Serum NO, (B) serum 5'-NT, (C) liver NO, (D) liver 5'-NT, and (E) liver MPO. Data are expressed as mean ± standard error of the mean (n = 5). Bars with different letters are statistically distinct at $p < 0.05$.

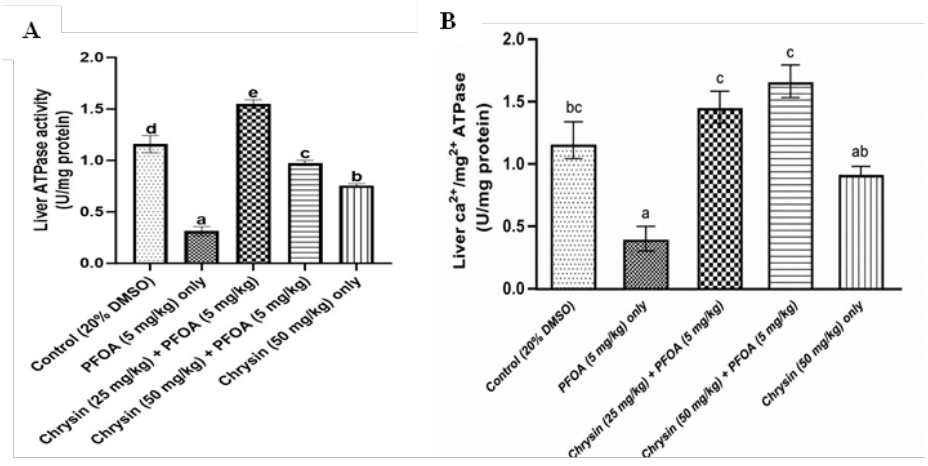


Figure 5. Effect of chrysin pre-treatment on membrane-bound enzyme activities in pentadecafluorooctanoic acid (PFOA)-induced hepatotoxicity in male Wistar rats. (A) Liver adenosine triphosphatase (ATPase). (B) Liver Ca^{2+}/Mg^{2+} ATPase. Data are expressed as mean ± standard error of the mean (n = 5). Bars with different letters are statistically distinct at $p < 0.05$.

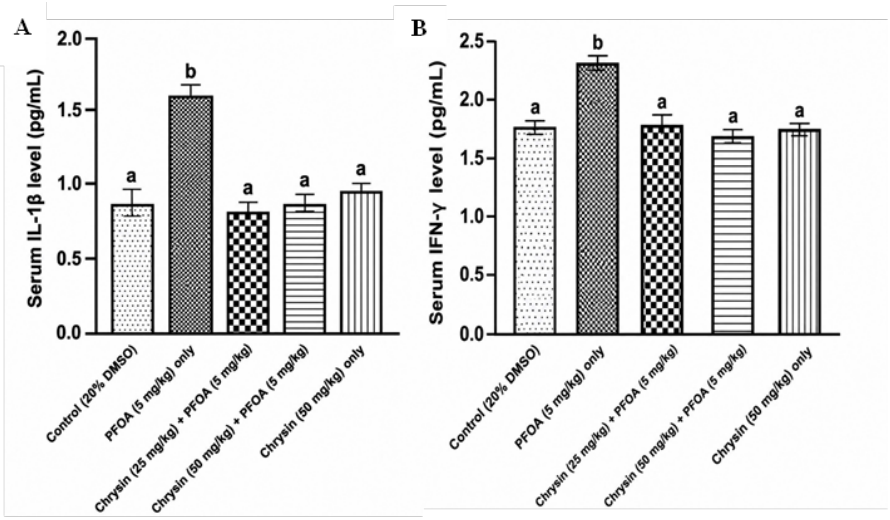


Figure 6. Effect of chrysin pre-treatment on pro-inflammatory cytokine levels in pentadecafluorooctanoic acid (PFOA)-induced hepatotoxicity in male Wistar rats. (A) Serum interleukin-1 beta. (B) Serum interferon gamma. Data are expressed as mean ± standard error of the mean (n = 5). Bars with different letters are statistically distinct at $p < 0.05$.

hepatoprotective effects in the present study by restoring the biochemical and metabolic alterations induced by PFOA exposure (Figures 1–6).

Pentadecafluorooctanoic acid exposure caused a significant ($p < 0.05$) increase in serum AST, ALT, and ALP activities compared to the control group (Figure 1), as supported by Gallo *et al.*[73] These enzymes are critical indicators of hepatocellular integrity and biliary function, with elevated levels suggesting hepatocellular damage and biliary obstruction.[74] PFOA's hepatotoxicity stems from its interference with membrane stability and mitochondrial function, leading to enzyme leakage into the bloodstream, thus resulting in a significant ($p < 0.05$) reduction in liver AST, ALT, and ALP activities.[75,76] This finding is consistent with reports by Darrow *et al.*[7] and Fenton *et al.*[77] Chrysin pre-treatment significantly ($p < 0.05$) reduced serum AST, ALT, and ALP activities while increasing the activities of these enzymes in the liver (Figure 1). The protective effect is attributed to chrysin's ability to stabilize hepatocyte membranes by reducing lipid peroxidation and to its antioxidant activity in protecting mitochondria from ROS-induced damage.[28,78]

The oxidative damage induced by PFOA was evidenced by a significant ($p < 0.05$) elevation of MDA levels (Figure 2A), which reflect oxidative degradation of membrane lipids due to excessive ROS production.[28,79,80] Concomitantly, key antioxidant markers such as reduced GSH, CAT, SOD, and GPx were significantly ($p < 0.05$) decreased (Figure 3A–3C), indicating impaired antioxidant defenses.[81] In addition, PFOA suppressed GST (Figure 3D), a crucial phase II detoxification enzyme that conjugates electrophilic toxins with GSH, thereby compromising the liver's detoxification capacity.[82] Chrysin demonstrated remarkable antioxidant properties by directly scavenging ROS and upregulating the nuclear factor erythroid 2-related factor 2 pathway, which promotes the expression of antioxidant genes.[83,84] This dual mechanism restored GSH levels, enhanced GST activity, and reactivated other key antioxidant enzymes, thereby reducing oxidative damage and maintaining cellular integrity.[28]

Pentadecafluorooctanoic acid exposure also caused significant disruptions in cellular energy metabolism.[10] LDH activity was significantly elevated ($p < 0.05$) in the serum and decreased in the liver (Figure 1D and 1F), reflecting cell membrane damage and a shift toward anaerobic glycolysis.[14] Furthermore, total ATPase and Ca^{2+}/Mg^{2+} ATPase activities were significantly suppressed ($p<0.05$) in the liver (Figure 5A and 5B), indicating compromised ion gradients and energy balance.[85] ATPases, including Na^{+}/K^{+}-ATPase, are essential for bile secretion and ion transport.[86,87] PFOA's interference with these enzymes contributes to hepatocellular dysfunction and bile flow obstruction.[88] Chrysin pre-treatment restored ATPase activities in a dose-dependent manner (25 and 50 mg/kg), thereby supporting bile secretion and preserving mitochondrial integrity.[89] The significant reduction in LDH activity ($p < 0.05$) upon chrysin treatment further indicated restoration of aerobic metabolism.[90]

The inflammatory cascade triggered by PFOA involves multiple interconnected pathways. PFOA induces oxidative

stress that activates the NLR family pyrin domain-containing 3 inflammasome in immune cells, leading to the cleavage and release of IL-1β and subsequent liver inflammation.[91,92] In addition, PFOA stimulates the toll-like receptor 4 pathway, which activates nuclear factor kappa B to enhance IL-1β transcription[93], and amplifies Janus kinase–signal transducer and activator of transcription signaling to increase IFN-γ production.[94] It also promotes macrophage polarization toward the pro-inflammatory M1 phenotype and enhances natural killer cell activity, further boosting IFN-γ secretion.[95–98] Elevated IL-1β not only recruits immune cells and amplifies inflammation but also activates hepatic stellate cells, promoting fibrosis and liver injury.[99] Chrysin countered these processes by significantly ($p < 0.05$) reducing IL-1β and IFN-γ levels through its anti-inflammatory and antioxidant properties (Figure 6).[100,101] It inhibited nuclear factor kappa B activation, suppressed Janus kinase–signal transducer and activator of transcription signaling, inhibited the NLR family pyrin domain-containing 3 inflammasome, and promoted a shift of macrophages from the M1 to the anti-inflammatory M2 phenotype.[28,102–105] Additionally, chrysin reduced MPO activity, indicating decreased neutrophil infiltration and a lower oxidative burst (Figure 4E).[24]

Furthermore, PFOA-induced metabolic disturbances extended to bile acid transport. PFOA decreased 5'-NT activity (Figure 4B and 4D), a sensitive marker for hepatobiliary dysfunction and cholestasis.[18–20] PFOA disrupts normal hepatobiliary function by inducing oxidative stress and inflammation, which impair the structural and functional integrity of hepatocytes and biliary epithelial cells.[106,107] Oxidative stress damages membrane-bound enzymes like 5'-NT, leading to its increased activity and release, while interference with bile acid transporters such as the bile salt export pump contributes to bile flow obstruction.[108–110] Chrysin pre-treatment significantly increased 5'-NT activity (Figure 4), restoring normal hepatobiliary function by protecting bile acid transporters and preserving membrane integrity.[109,111,112]

Administration of PFOA also resulted in a significant ($p < 0.05$) reduction in hepatic NO levels (Figure 4A and 4C). NO, a key signaling molecule regulating hepatic blood flow and immune responses, competes with oxygen at the binding site of cytochrome oxidase, thereby influencing oxygen consumption and ATP production.[113,114] Reduced NO levels impair vasodilation, contribute to hypoxia, and limit the neutralization of ROS.[22,115] Accumulation of ROS promotes peroxynitrite formation, exacerbating oxidative stress and inflammation.[116] Bogdan[117] has shown that diminished NO levels drive macrophages toward the M1 pro-inflammatory phenotype, further elevating IL-1β and IFN-γ levels. Chrysin pre-treatment significantly ($p < 0.05$) restored NO levels by reducing oxidative stress and enhancing NO synthase activity, thereby improving NO bioavailability and mitigating hypoxia and inflammation.[118]

While the observed reductions in serum ALT and AST following chrysin pre-treatment were statistically significant (Figure 1A and 1B), their toxicological relevance should be interpreted cautiously. Serum aminotransferase activities should not be considered in isolation as definitive evidence of overt hepatocellular necrosis.[119] In rodent studies, modest elevations in ALT that remain below approximately 50 U/L (or less than a two- to three-fold increase above baseline) generally reflect early hepatocellular injury, subclinical metabolic adaptation, or reversible membrane perturbation rather than extensive irreversible tissue necrosis.[119–125] Consistent with this interpretation, PFOA exposure significantly increased serum ALT and AST while decreasing their corresponding hepatic activities, indicating leakage of intracellular enzymes following hepatocellular membrane injury. However, the magnitude of these changes remained below levels typically associated with severe hepatocellular necrosis. Importantly, these alterations occurred concurrently with increased oxidative stress, inflammation, membrane dysfunction, and metabolic disturbances, collectively providing strong biochemical evidence of PFOA-induced hepatic injury. Chrysin pre-treatment significantly attenuated these abnormalities by reducing serum aminotransferase activities while restoring hepatic ALT and AST activities toward normal levels, suggesting preservation of hepatocyte membrane integrity, reduced enzyme leakage, and maintenance of intracellular enzyme localization. Therefore, the hepatoprotective effects of chrysin appear to involve prevention of early hepatocellular injury and preservation of hepatic cellular homeostasis, thereby limiting progression toward more severe liver damage.

Collectively, these findings demonstrate that oxidative stress, inflammation, and metabolic dysfunction are closely interconnected in the pathogenesis of PFOA-induced hepatotoxicity, and that chrysin exerts dose-dependent hepatoprotective effects through simultaneous modulation of these processes.[68,77] The present findings are consistent with previous reports describing the potent antioxidant, anti-inflammatory, anti-apoptotic, and hepatoprotective properties of chrysin; however, our study extends current knowledge by providing a comprehensive biochemical evaluation of its protective effects against PFOA-induced liver injury.[32,69] Unlike many naturally occurring hepatoprotective flavonoids, such as quercetin and silymarin, which have primarily been investigated for

individual antioxidant or anti-inflammatory mechanisms, chrysin appears to exert broader protective effects by simultaneously restoring oxidative balance, attenuating inflammatory responses, preserving membrane integrity, and improving metabolic homeostasis.[32,76,126] Although the involvement of molecular pathways such as the nuclear factor erythroid 2-related factor 2/heme oxygenase-1 axis and NLR family pyrin domain-containing 3 inflammasome remains speculative in the present study because these pathways were not directly investigated, the observed biochemical profile is consistent with mechanisms previously reported in experimental models of chrysin-mediated hepatoprotection.[32,69] These findings further support the therapeutic potential of chrysin as a promising natural flavonoid for mitigating per- and polyfluoroalkyl substance-induced hepatotoxicity and provide a biochemical foundation for future molecular and translational investigations.

Although chrysin demonstrated dose-dependent hepatoprotective effects at oral doses of 25 and 50 mg/kg in the present study, its relatively poor systemic bioavailability should be considered when interpreting these findings. Pharmacokinetic studies have shown that orally administered chrysin undergoes extensive intestinal and hepatic first-pass metabolism through glucuronidation and sulfation, resulting in low circulating concentrations despite relatively high administered doses.[69,72] Nevertheless, experimental studies have consistently demonstrated biological activity within this dose range, suggesting that repeated dosing, tissue distribution, local hepatic exposure, and active metabolites (including glucuronide and sulfate conjugates) may contribute to its observed pharmacological effects.[28,32,127–136] Therefore, although the present findings support the hepatoprotective potential of chrysin, its poor oral bioavailability should be considered when translating these doses to clinical settings, highlighting the need for formulation strategies, such as nanoencapsulation, liposomal delivery systems, or prodrug approaches, to improve systemic exposure.[137]

Despite the comprehensive biochemical evaluation conducted in this study, several limitations should be acknowledged. First, the relatively small sample size ($n = 5$ per group) may have limited the statistical power, although consistent treatment-related effects were observed across multiple independent biomarkers of hepatic function, oxidative stress, inflammation, membrane integrity, and energy metabolism. Second, histopathological examination of liver tissue was not performed. While the extensive biochemical findings collectively provide strong evidence of PFOA-induced hepatocellular injury and the hepatoprotective effects of chrysin, histopathological assessment would have provided important morphological confirmation of these observations.[32] In addition, the proposed involvement of molecular pathways, including nuclear factor erythroid 2-related factor 2/heme oxygenase-1 activation and suppression of nuclear factor kappa B, Janus kinase–signal transducer and activator of transcription signaling, and the NLR family pyrin domain-containing 3 inflammasome, was inferred from previous experimental studies and the observed biochemical responses rather than being directly evaluated using molecular techniques such as Western blotting, target-specific enzyme-linked immunosorbent assay or quantitative polymerase chain reaction. Furthermore, the 14-day exposure model represents an acute experimental paradigm that may not fully reflect the chronic, low-dose human exposure typically associated with environmental PFOA contamination.[77] Finally, only male Wistar rats were included in this study. Although this approach minimized hormonal variability associated with the estrous cycle, sex-related differences in PFOA toxicokinetics, hepatic metabolism, inflammatory responses, antioxidant defenses, and liver enzyme activities have been reported, suggesting that females may exhibit distinct biochemical responses to PFOA exposure.[138,139]

Future studies should address these limitations by employing larger sample sizes, chronic exposure models that better mimic environmental PFOA exposure, and the inclusion of both male and female animals to clarify sex-dependent differences in hepatotoxicity and chrysin responsiveness. Integrating histopathological assessment with molecular approaches, including Western blotting, quantitative polymerase chain reaction, and immunohistochemistry, would enable direct validation of the proposed nuclear factor erythroid 2-related factor 2/heme oxygenase-1, nuclear factor kappa B, and NLR family pyrin domain-containing 3 inflammasome signaling pathways and strengthen the mechanistic basis of chrysin-mediated hepatoprotection. In addition, strategies to improve the poor oral bioavailability of chrysin, such as nanoencapsulation, liposomal delivery systems, or prodrug formulations, should be explored to enhance its therapeutic potential.[69,112] Further investigation into its interactions with nuclear receptors, epigenetic regulation, and the gut–liver axis may also provide valuable insights into the development of flavonoid-based interventions against per- and polyfluoroalkyl substance-induced liver injury. Nevertheless, the present findings provide a strong biochemical foundation for understanding PFOA-induced hepatotoxicity and support the continued investigation of chrysin as a promising natural hepatoprotective agent against per- and polyfluoroalkyl substance-associated liver injury.

5. Conclusion

This study demonstrates that PFOA induces biochemical alterations consistent with hepatocellular injury, characterized by oxidative stress, inflammation, membrane dysfunction, and metabolic disturbances, resulting in impaired hepatic function in male Wistar rats. These alterations were evidenced by increased serum liver injury biomarkers, enhanced lipid peroxidation, depletion of endogenous antioxidant defenses, disruption of membrane-bound enzyme activities, altered energy metabolism, and elevated pro-inflammatory mediators. Chrysin pre-treatment (25 and 50 mg/kg) significantly and dose-dependently attenuated these biochemical abnormalities by restoring hepatic antioxidant capacity, reducing oxidative stress and inflammatory responses, preserving membrane integrity, improving metabolic homeostasis, and normalizing liver function biomarkers. Collectively, these findings demonstrate the hepatoprotective potential of chrysin against PFOA-induced liver injury and support its promise as a natural therapeutic candidate for mitigating per- and polyfluoroalkyl substance-induced hepatotoxicity. Nevertheless, the absence of histopathological and molecular validation, together with the acute exposure model and the exclusive use of male animals, warrants cautious interpretation of these findings. Future studies employing chronic exposure models, both sexes, histopathological and molecular analyses, and improved chrysin delivery strategies are needed to further elucidate its mechanisms of action and enhance its translational potential.

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Conflict of interest

The author declares no conflicts of interest.

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Ethics approval and consent to participate

Ethical approval was obtained from the Animal Ethics Committee of the Federal University of Agriculture, Abeokuta (Approval No. FUNAAB/COLBIOS/BCH/UG/20201184).

Consent for publication

Not applicable.

Availability of data

Data will be made available upon request to the corresponding author.

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