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RESEARCH ARTICLE

Ameliorative effects of eugenol and naringenin on perfluorooctanoic acid-induced hepatotoxicity in Sprague-Dawley rats

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Abstract

Perfluorooctanoic acid (PFOA), an environmental pollutant with wide industrial usage, is known to induce hepatotoxicity through oxidative stress, inflammation and lipid dysregulation. This study evaluated the hepatoprotective effects of eugenol and naringenin against PFOA–induced liver damage in Sprague-Dawley rats. Hepatotoxicity was induced via the oral administration of PFOA (40 mg/kg) for 21 days. Simultaneously, the rats were treated with eugenol (5 and 10 mg/kg) and naringenin (10 and 50 mg/kg). Serum biochemical parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol and triglycerides, liver thiobarbituric acid reactive substances (TBARS) levels and histopathological changes were assessed. *In silico* molecular docking was performed to explore the interactions of the test compounds with adiponectin. PFOA significantly elevated serum transaminases, lipid parameters and hepatic TBARS levels and induced histological changes such as steatosis, necrosis and inflammation. Treatment with eugenol and naringenin significantly ($P < 0.05$) mitigated these alterations, particularly at higher doses. Molecular docking revealed favourable binding affinities of both compounds with adiponectin, suggesting a mechanistic role in their hepatoprotective effects. Eugenol and naringenin exerted protective effects against PFOA-induced hepatotoxicity through antioxidant, anti–inflammatory and lipid–modulating actions, possibly via adiponectin signalling pathways. These findings highlight their therapeutic potential in managing environmentally induced liver damage.

Keywords: adiponectin; antioxidants; eugenol; hepatotoxicity; naringenin; perfluorooctanoic acid

Introduction

Perfluorooctanoic acid (PFOA) is a synthetic perfluorinated compound extensively used in various industrial applications, including the manufacture of nonstick cookware, water-repellent fabrics and fire-fighting foams. Owing to its chemical stability and resistance to degradation, PFOA persists in the environment and bioaccumulates in living organisms, raising substantial environmental and health concerns. Epidemiological and toxicological studies have linked PFOA exposure to hepatotoxic effects, including elevated liver enzymes, lipid dysregulation and the development of non-alcoholic fatty liver disease (NAFLD) (1). The liver, a vital organ responsible for detoxification, metabolism and biochemical synthesis, is particularly susceptible to xenobiotic–induced injury. Hepatotoxicity may result from diverse exposures, including exposure to pharmaceuticals, alcohol and environmental pollutants, leading to significant liver dysfunction and chronic liver disease. Among these, NAFLD is increasingly recognised as a global health burden, with increasing incidence driven by obesity and metabolic syndrome. Environmental pollutants such as PFOA have been implicated in exacerbating NAFLD pathogenesis by disrupting lipid homeostasis and inducing oxidative stress (1).

In recent years, considerable attention has been given to naturally derived phytochemicals capable of mitigating toxin-induced liver injury. Naringenin, a flavonoid abundant in citrus fruits and eugenol, a phenolic compound derived from clove oil, have emerged as promising hepatoprotective agents. Naringenin is known to modulate lipid metabolism via peroxisome proliferator activated receptor (PPAR) activation, reduce the hepatic lipid accumulation and suppress inflammation through the downregulation of nuclear factor-kappa B (NF-κB) signalling (2). Eugenol exerts potent antioxidant and anti-inflammatory effects by scavenging free radicals and inhibiting lipid peroxidation, thus preserving cellular integrity (3).

To understand the molecular basis of their hepatoprotective activity, the present study employed computational docking techniques targeting adiponectin, a key regulatory adipocytokine involved in glucose and lipid metabolism. Adiponectin enhances hepatic insulin sensitivity, promotes fatty acid oxidation and attenuates inflammatory signalling. Reduced adiponectin activity is strongly associated with steatosis, insulin resistance and liver dysfunction (4). Mechanistically, adiponectin activates AMP-activated protein kinase (AMPK), which in turn regulates lipid

metabolism and energy homeostasis (5). Molecular docking of eugenol and naringenin with adiponectin was conducted to assess their potential interactions and to elucidate the mechanistic rationale for their protective effects against PFOA-induced hepatic toxicity. Accordingly, this study was designed to evaluate the protective efficacy of eugenol and naringenin against PFOA-induced hepatotoxicity in Sprague-Dawley rats via an integrated approach involving biochemical, histopathological and computational methodologies.

Materials and Methods

Test substances

PFOA (95 %, 335-67-1) was procured from M/s Otto Chemie Pvt. Ltd, Maharashtra, India. Naringenin (67604-48-2) and eugenol (97-53-0) were purchased from M/s Sisco Research Laboratories Pvt. Ltd (SRL), Maharashtra, India. Silymarin (S0292-10G) was procured from M/s Sigma Aldrich, Bangalore, India. They were stored in the refrigerator until use for the experimental study.

Chemicals/drugs/diagnostic kits/instruments

All the chemicals used were of analytical grade procured from M/s Otto chemie, M/s Sigma-Aldrich and M/s SRL, India. The diagnostic kits were procured from M/s Agappe Diagnostics Ltd., India. The major instruments used for the study were a semiautomatic biochemical analyser (M/s Hospitex, Italy) and a UV-VIS/NIR Spectrophotometer (M/s PerkinElmer, Singapore).

Experimental design

The experimental protocol was approved by the Institutional Animal Ethics Committee of the College of Veterinary and Animal Sciences, Mannuthy (Approval No. CVAS/MTY/IAEC/24/67). Forty two female Sprague-Dawley rats (8–12 weeks old), weighing between 150 and 200 g, were randomly divided into seven groups, with six animals in each group. Each group received the respective treatment orally in 0.1 % dimethyl sulfoxide (DMSO) for 21 days. The test compound, perfluorooctanoic acid (PFOA), was solubilised in 0.1 % DMSO and administered orally at a dose of 40 mg/kg at 24 hr intervals for 21 consecutive days (6). Silymarin was used as a standard drug at a 50 mg/kg dosage (7). Eugenol and naringenin were administered concurrently with PFOA orally for 21 days. Eugenol (groups 4 and 5) and naringenin (groups 6 and 7) were administered in two doses at a dose rate of 5 and 10 mg/kg for eugenol while 10 and 50 mg kg^{-1} for naringenin (8–10). The animals were fasted overnight before dosing. Hepatic function was assessed on days 0 and 22 through the estimation of serum ALT, AST, total cholesterol and triglyceride levels using standard diagnostic kits. After the 21–day treatment period, the animals were euthanised according to the ethical guidelines and the livers were collected. Liver samples were used for the estimation of the TBARS assay (11). Representative samples were also collected for histopathological examination (12). The detailed methodology is provided in Supplementary File.

In silico docking

The docking investigation was conducted using AutoDock V4.2. The ligands were modified using MarvinView 17.25.0. The conversion of chemical file formats was performed using Open Babel 3.1.1. Accelrys discovery studio was used to visualise the docked conformations and version 2.2.8 of Ligplot+ was used to visualise the hydrophobic interactions of ligands with the receptor (13).

The macromolecule adiponectin was downloaded from the RCSB protein data bank (pdb id: 5LWY) in PDB format, which was further converted to PDBQT format (Fig. 1). The chemical structures of the compounds were downloaded in the spatial data file (.SDF) format from the National Centre for Biotechnology Information's PubChem Compound Database (https://pubchem.ncbi.nlm.nih.gov/). Because orally active therapeutics are typically small molecules, the drug–likeness of the test ligands was considered using Lipinski's Rule of Five (molecular weight <= 500 Da, logP <= 5, hydrogen bond donors <= 5 and hydrogen bond acceptors <= 10) as a standard interpretive framework alongside docking. The structures of naringenin (439246), eugenol (3314), silymarin (5213) and PFOA (9554) were modified in MarvinView and then converted to Tripos Mol 2 file format (Fig. 2). The AutoDock tool's ligand modification capabilities were utilised to construct ligand structures for root expansion, root identification and rotatable bond count selection. Following the first preparation, the ligands were transformed into PDBQT format. The adiponectin receptor was docked with each of the four ligands using

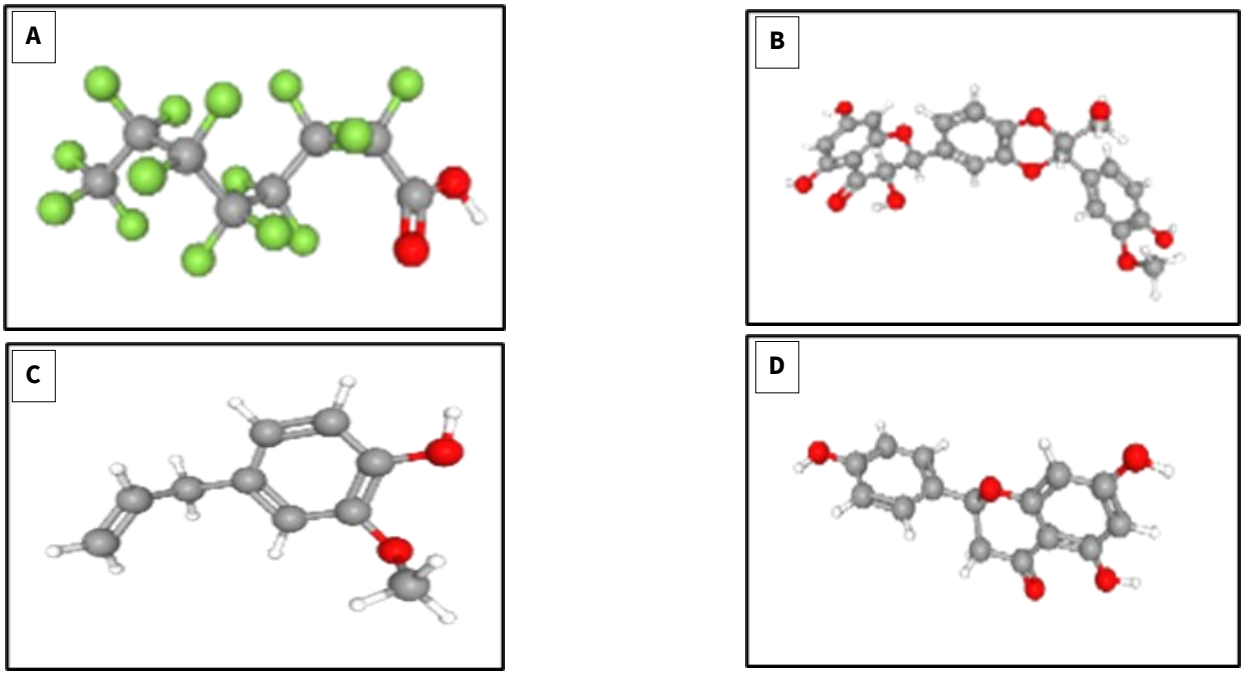

Fig. 1. Ligands used: A) PFOA, B) Silymarin, C) Eugenol, D) Naringenin.

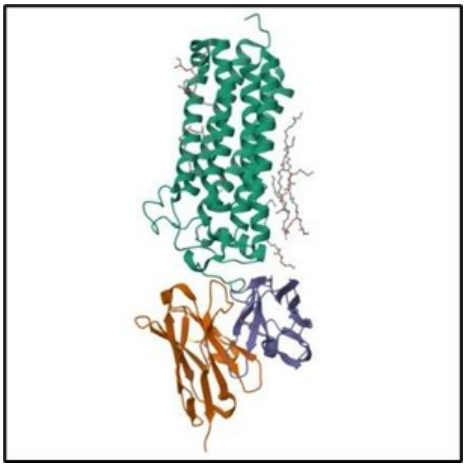

Fig. 2. Structure of receptor protein: adiponectin.

Autodock V4.2. Every docking run was conducted using the Lamarckian genetic method. Together with the RMSD table, the binding energy was extracted from the dlg file. Next, the binding energies (kcal mol^{-1}) were evaluated. The docking analysis results were visualised. The most energetically advantageous conformations of each ligand were chosen after examining how each ligand interacted with the protein and examining its binding patterns. Using Ligplot+ version v.2.2.8, the hydrophobic interactions with the ligand and receptor protein were visualised.

Statistical analysis

The data from the animal study were analysed statistically via IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp. (2016). The results were expressed as mean ± SE. The statistical significance was measured at $P < 0.05$.

Results

The mean body weights of all seven groups on days 0, 14 and 22 are presented in Table 1. The results revealed an increase in body weight across all groups, which was not statistically significant. The mean ALT values for all the seven groups on days 0 and 22 are presented in Table 2. On day 0, no significant ($P > 0.05$) difference in the mean serum ALT values were observed between the groups. However, on day 22, a significant ($P < 0.05$) increase in the mean serum ALT level was observed in the PFOA–treated group 2 animals, with a mean value of 80.81 ± 2.67 IU L^{-1}. On the same day, a significant ($P < 0.05$) decrease in the mean serum ALT values was noted in groups 3 to 7. The reduction in ALT values in groups 5 to 7 was statistically comparable to that in group 1.

Table 1. Effect of eugenol and naringenin on body weight in PFOA-treated Sprague-Dawley rats

Groups	Body weight (g)		
	Day 0	Day 14	Day 22
1	171.66 ± 2.06	178.85 ± 5.56	190.66 ± 3.17
2	175.00 ± 4.04	201.667 ± 3.27	218.33 ± 2.60
3	197.00 ± 3.00	206.66 ± 5.69	216.00 ± 3.46
4	182.00 ± 4.35	200.66 ± 2.96	208.33 ± 0.88
5	174.00 ± 4.72	185.00 ± 4.04	207.00 ± 4.04
6	179.00 ± 2.08	194.00 ± 4.16	213.00 ± 3.88
7	173.33 ± 3.84	187.33 ± 3.48	195.33 ± 4.70

The values are expressed as the means ± SEs, n= 6; means bearing the same superscripts do not differ significantly at $P < 0.05$.

Table 2. Effect of eugenol and naringenin on ALT levels in PFOA-treated Sprague-Dawley rats

Groups	Alanine aminotransferase (IU L^{-1})	
	Day 0	Day 22
1	66.89 ± 3.61	66.55 ± 2.14^{a}
2	60.35 ± 2.32	80.81 ± 2.67^{ab}
3	57.20 ± 3.24	53.69 ± 4.68^{ab}
4	52.12 ± 4.85	57.65 ± 3.40^{ab}
5	62.43 ± 2.17	67.99 ± 3.12^{a}
6	58.26 ± 2.05	60.46 ± 1.74^{a}
7	62.01 ± 3.86	71.27 ± 2.21^{a}

The values are expressed as the means ± SEs, n= 6; means bearing the same superscripts do not differ significantly at $P < 0.05$.

The mean AST values for all the seven groups on days 0 and 22 are presented in Table 3. On day zero, there was no significant ($P > 0.05$) difference in the mean serum AST levels among the groups. On day 22, a significant ($P < 0.05$) increase in the mean serum AST level was recorded in the PFOA-treated group 2 animals, with a mean value of 168.63 ± 0.87 IU/L. The mean AST levels of groups 3 to 7 on day 22 were statistically comparable with those of group 1. The mean total cholesterol values for all the seven groups on days 0 and 22 are presented in Table 4. On day zero, there was no significant ($P > 0.05$) difference in total serum cholesterol levels among the groups. On day 22, the mean cholesterol level increased significantly ($P < 0.05$) in the PFOA–treated group, with a mean value of 86.46 ± 1.82 mg/dL. A significant ($P < 0.05$) reduction in the mean serum cholesterol level was observed in groups 3 to 7, with mean values of 55.22 ± 2.96, 54.20 ± 2.26, 51.21 ± 4.36, 53.58 ± 2.24 and 43.90 ± 0.58 mg/dL, respectively.

Table 3. Effect of eugenol and naringenin on AST levels in PFOA-treated Sprague-Dawley rats

Groups	Aspartate aminotransferase (IU/L)	
	Day 0	Day 22
1	114.33 ± 2.60	140.73 ± 2.01^{a}
2	122.100 ± 2.66	168.63 ± 0.87^{b}
3	110.36 ± 1.60	140.26 ± 2.08^{ac}
4	126.50 ± 3.98	128.26 ± 1.32^{ac}
5	114.53 ± 2.84	137.66 ± 1.45^{ac}
6	111.62 ± 2.23	131.20 ± 1.05^{ac}
7	110.63 ± 3.75	134.83 ± 2.45^{ac}

The values are expressed as the means ± SEs, n= 6; means bearing the same superscripts do not differ significantly at $P < 0.05$.

Table 4. Effects of eugenol and naringenin on total serum cholesterol in PFOA-treated Sprague-Dawley rats

Groups	Total cholesterol (mg/dL)	
	Day 0	Day 22
1	49.82 ± 2.15	69.19 ± 1.72^{a}
2	54.99 ± 1.07	86.46 ± 1.82^{b}
3	47.60 ± 1.44	55.22 ± 2.96^{bc}
4	50.03 ± 1.44	54.20 ± 2.26^{bc}
5	41.96 ± 5.60	51.21 ± 4.36^{bc}
6	48.53 ± 0.74	53.58 ± 2.24^{bc}
7	40.89 ± 2.10	43.90 ± 0.58^{bc}

The values are expressed as the means ± SEs, n= 6; means bearing the same superscripts do not differ significantly at $P < 0.05$.

The mean serum triglyceride values for all the seven groups on days 0 and 22 are presented in Table 5. On day zero the serum triglyceride levels were within the normal range and no significant ($P > 0.05$) differences in the mean values were observed among the groups. On day 22, the mean serum triglyceride level increased significantly ($P < 0.05$) in the PFOA–treated group, with a mean value of 68.36 ± 1.58 mg/dL. A significant reduction in serum triglyceride levels was observed in groups 3 to 7, with mean values of 40.57 ± 2.31, 44.92 ± 1.92, 50.60 ± 1.22, 55.46 ± 3.04 and 55.25 ± 2.25 mg/dL, respectively. The mean TBARS concentrations for all the seven groups on days 0 and 22 are presented in Table 6. The mean TBARS level in liver tissue was elevated in the PFOA–treated group, with a mean value of 81.06 ± 2.16 mM/100 mg. A significant ($P < 0.05$) reduction in mean TBARS levels was observed in liver tissues treated with silymarin, eugenol and naringenin, with mean values of 60.03 ± 0.83, 63.18 ± 0.97, 65.70 ± 2.71, 64.81 ± 2.92 and 66.93 ± 0.63 mM/100 mg, respectively. The mean TBARS concentrations for all seven groups on days 0 and 22 are presented in Table 6.

Table 5. Effect of eugenol and naringenin on serum triglycerides in PFOA–treated Sprague-Dawley rats

Groups	Triglycerides (mg/dL)	
	Day 0	Day 22
1	52.85 ± 2.80	48.50 ± 2.27^{a}
2	58.38 ± 0.81	68.36 ± 1.58^{b}
3	60.64 ± 2.67	40.57 ± 2.31^{ac}
4	50.01 ± 0.90	44.92 ± 1.92^{ac}
5	52.95 ± 1.38	50.60 ± 1.22^{a}
6	49.75 ± 4.91	55.46 ± 3.04^{a}
7	55.32 ± 2.78	55.25 ± 2.25^{a}

The values are expressed as the means ± SEs, n= 6; means bearing the same superscripts do not differ significantly at $P < 0.05$.

Table 6. Effect of eugenol and naringenin on TBARS in PFOA-treated Sprague-Dawley rats

Groups	Thiobarbituric acid reactive substances, (mM/100 mg)
1	52.76 ± 1.15^{a}
2	81.06 ± 2.16^{b}
3	60.03 ± 0.83^{ac}
4	63.18 ± 0.97^{bc}
5	65.70 ± 2.71^{bc}
6	64.81 ± 2.92^{bc}
7	66.93 ± 0.63^{bc}

The values are expressed as the means ± SEs, n= 6; means bearing the same superscripts do not differ significantly at $P < 0.05$.

Histopathological parameters such as steatosis, ballooning degeneration, inflammatory cell infiltration, necrosis and single-cell necrosis were recorded. Microscopic examination of liver sections from the normal control group revealed normal hepatic architecture, with hepatocyte cords radiating from the central vein (Fig. 3). Liver sections from PFOA-treated animals showed mild to moderate microvesicular steatosis (Fig. 4). There was no to mild inflammatory cell infiltration, consisting predominantly of mononuclear cells and ballooning degeneration was absent. In addition, single-cell necrosis and minimal necrosis of hepatocytes were observed (Fig. 5–6). Treatment with silymarin resulted in no to mild microvesicular steatosis (Fig. 7). Inflammatory cell infiltration and necrosis were absent; however, single-cell necrosis was observed in two animals (Fig. 8). In the low-dose eugenol-treated group, the liver showed no to mild steatosis with no to minimal inflammatory cell infiltration and necrosis (Fig. 9). In the high-dose eugenol–treated group, necrosis, steatosis and single-cell necrosis were absent. The liver exhibited significant restoration of normal architecture (Fig. 10), with only minimal inflammatory cell infiltration. Histopathological examination of the low–dose naringenin–treated group revealed mild steatosis and ballooning degeneration, with minimal inflammatory cell infiltration and evidence of single-cell necrosis (Fig. 11). In the high-dose naringenin-treated group, steatosis, ballooning degeneration and necrosis were absent. However, single-cell necrosis and minimal inflammatory cell infiltration were still observed (Fig. 12).

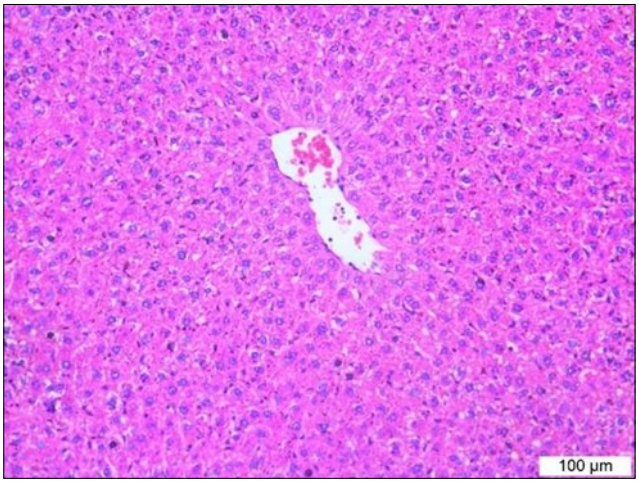

Fig. 3. Histological sections of liver tissue showing normal architecture of liver with hepatocyte cords radiating from the central vein (H&E, X200).

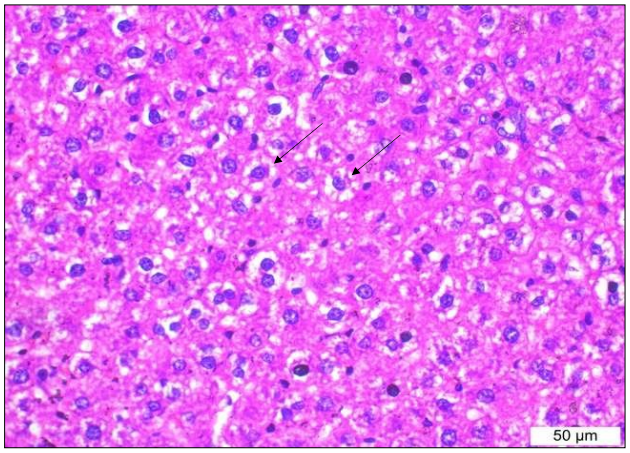

Fig. 4. PFOA–induced liver damage showing moderate microvesicular steatosis–multiple small vacuoles in hepatocytes (H&E, X400).

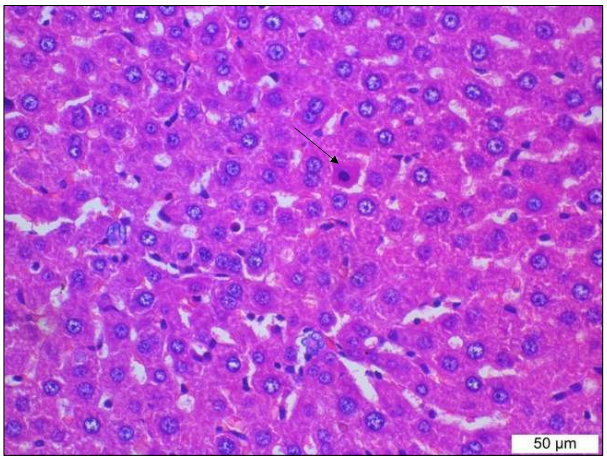

Fig. 5. PFOA–induced liver damage showing single cell necrosis with increased eosinophilic staining of cytoplasm and pyknotic nucleus (H&E, X400).

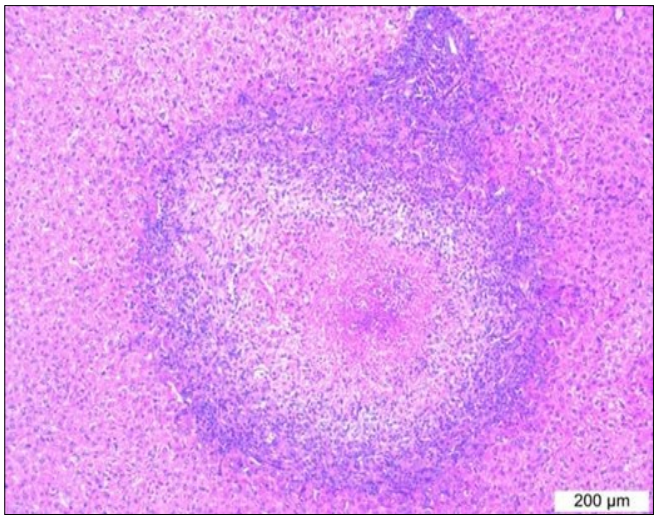

Fig. 6. PFOA–induced liver damage showing focal necrotic area and inflammatory cell infiltration (H&E, X100).

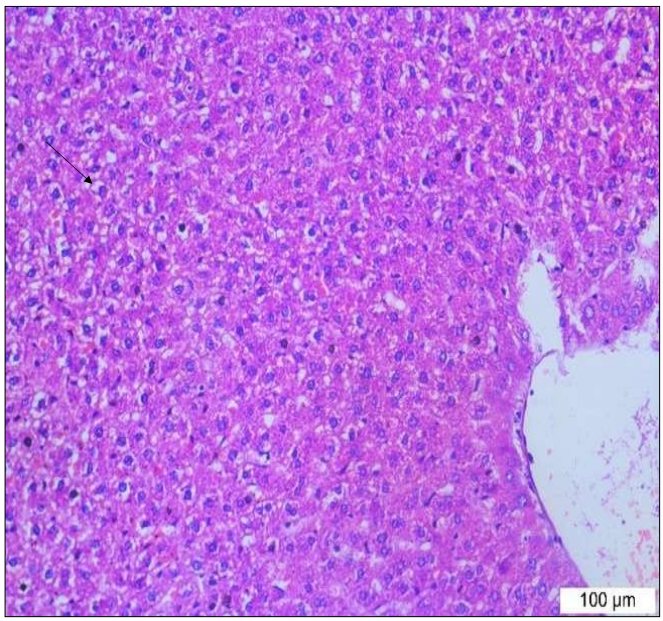

Fig. 7. Hepatoprotective effects of silymarin showing mild microvesicular steatosis– multiple small vacuoles in hepatocytes (H&E, X200).

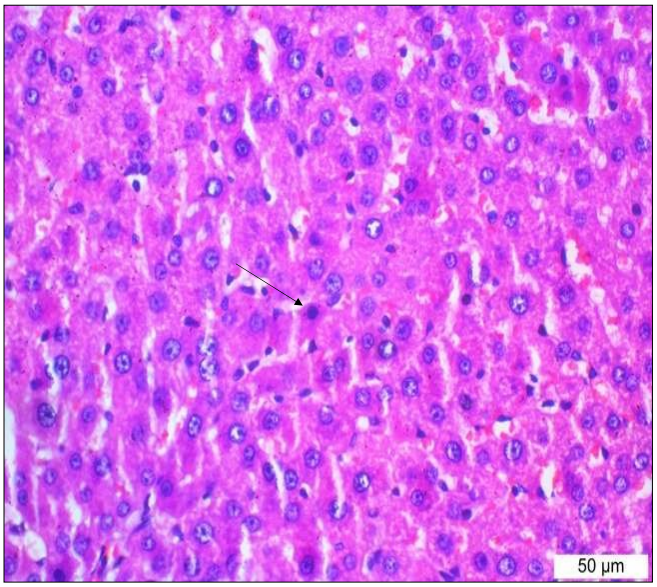

Fig. 8. Hepatoprotective effects of silymarin showing single cell necrosis with increased eosinophilic staining of cytoplasm and pyknotic nucleus (H & E, X400).

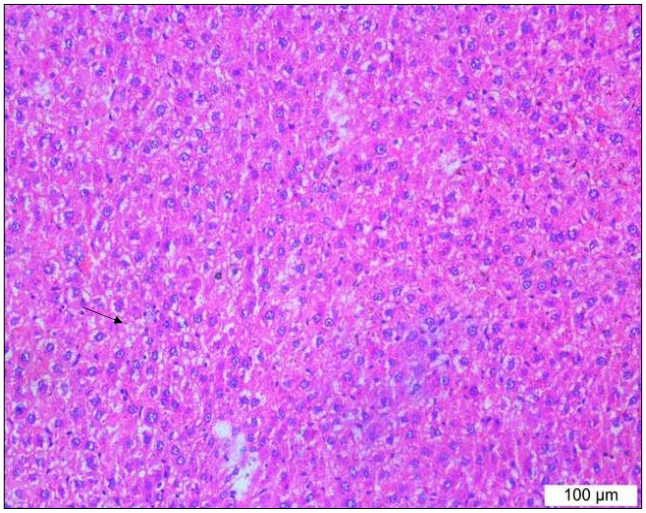

Fig. 9. Hepatoprotective effects of eugenol at low dose showing mild microvesicular steatosis–multiple small vacuoles in hepatocytes (H & E, X200).

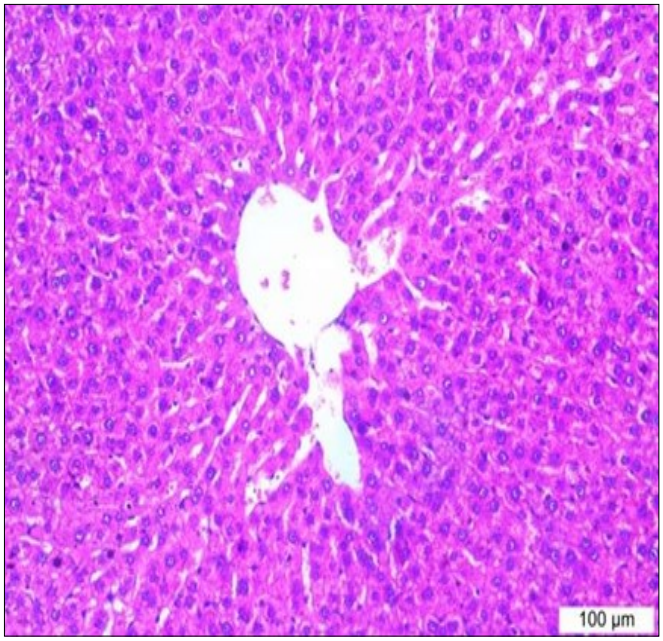

Fig. 10. Hepatoprotective effects of eugenol at high dose showing restoration of normal architecture with no steatosis (H & E, X200).

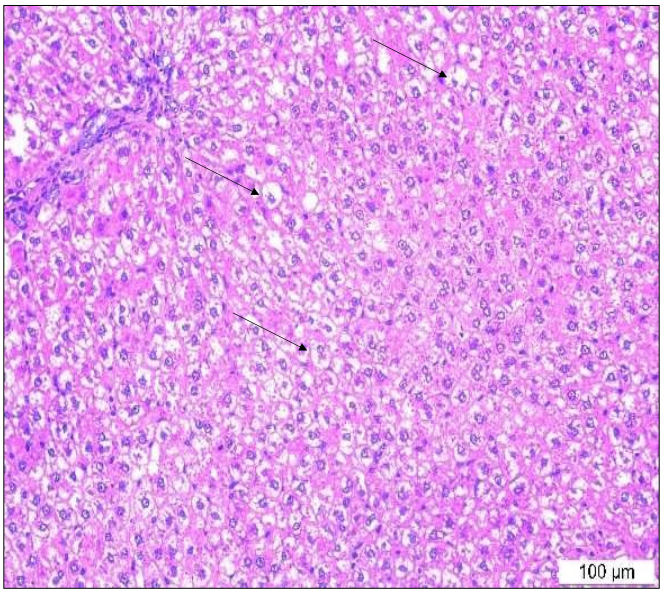

Fig. 11. Hepatoprotective effects of naringenin at low dose showing mild ballooning degeneration (H&E, X200).

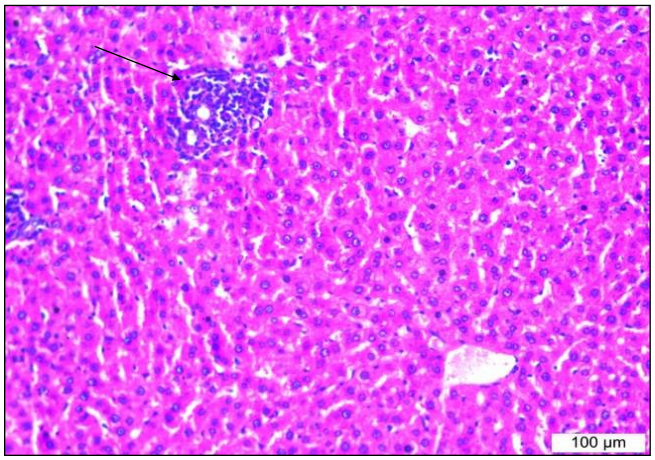

Fig. 12. Hepatoprotective effects of naringenin at high dose showing minimal mononuclear cell infiltration (H & E, X200).

The binding energies of the ligands and standard drug obtained from the RMSD table were tabulated (Table 7). PFOA showed a low binding affinity (-5.6 kcal mol^{-1}) for adiponectin, with minimum hydrophobic interactions and few/no hydrogen bonds. In contrast, naringenin showed a binding affinity of -6.0 kcal/mol with moderate hydrophobic interactions and a few hydrogen bonds with active site residues, whereas eugenol showed a binding affinity of -6.5 kcal/mol and engaged more hydrophobic residues and likely formed hydrogen bonds. Silymarin had the highest binding affinity of -8.0 kcal/mol and demonstrated the strongest hydrophobic interactions and the highest number of hydrogen bonds. The results of the hydrophobic interactions and hydrogen bonds are depicted in Fig. 13.

Table 7. Binding energy of different ligands and standard drugs with adiponectin (5LWY)

No.	Ligands	Binding energy
1	Perfluorooctanoic acid	-5.6 kcal/mol
2	Eugenol	-6.5 kcal/mol
3	Naringenin	-6 kcal/mol
4	Silymarin	-8.0 kcal/mol

Discussion

The present study investigated the potential of naringenin and eugenol to ameliorate PFOA-induced hepatotoxicity in Sprague Dawley rats through a comprehensive approach that included biochemical, histological and *in silico* analyses. The PFOA, which is extensively used in various industrial applications due to its chemical stability, is known to accumulate in the liver following oral or inhalation exposure, where it exerts hepatotoxic effects such as dyslipidemia and steatosis by disrupting lipid metabolism and inducing oxidative stress (14, 15). Throughout the 21-day study period, the body weight changes were not statistically significant across any treatment groups, suggesting that the administered PFOA dose (40 mg/kg) did not cause systemic toxicity severe enough to impact the growth of the animals. Research indicates that no significant alterations in body weight following PFOA exposure at similar doses (16). Additionally, the absence of adverse effects on body weight in the groups treated with eugenol and naringenin corroborates their metabolic safety profiles, as highlighted in earlier reports (17, 18).

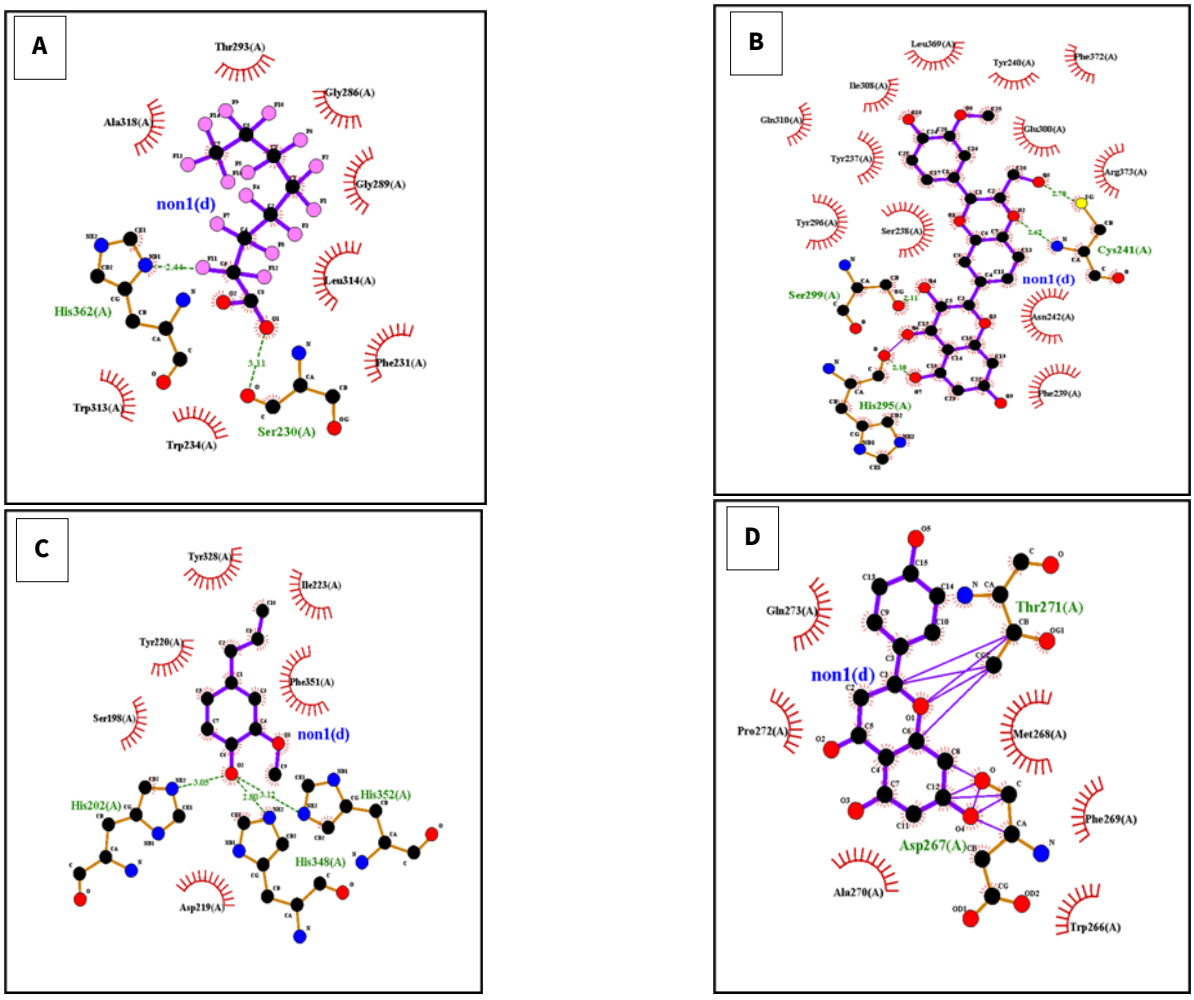

Fig. 13. Hydrophobic interactions and hydrogen bonding between ligands and receptor. A) PFOA, B) Naringenin, C) Eugenol, D) Silymarin

Biochemical analyses demonstrated significant increase in serum ALT and AST levels in the PFOA–treated group, indicating hepatic injury and hepatocellular damage. Elevated transaminases are well-established markers of liver damage and reflect the leakage of these enzymes from damaged hepatocytes. The significant attenuation of these enzyme levels in groups co–treated with silymarin, naringenin, or eugenol provides strong evidence of their hepatoprotective efficacy. These findings are consistent with the known hepatoprotective, anti–inflammatory and antioxidant properties of these phytochemicals (19, 20). Serum lipid profile analyses further confirmed the hepatotoxic impact of PFOA exposure, with marked increases in total cholesterol and triglyceride levels. Such alterations reflect the disruption of lipid metabolism by PFOA, likely through its interference with key regulatory pathways involved in lipid synthesis, transport and clearance. The significant reductions in these parameters upon treatment with naringenin and eugenol indicate their ability to modulate lipid homeostasis and counteract the dyslipidemic effects of PFOA. Previous research supports this role, showing that both compounds can effectively regulate lipid metabolism and decrease lipid levels in models of hyperlipidemia (21, 22). Lipid peroxidation, as indicated by the elevated TBARS levels in the liver tissues of PFOA–treated animals, underscores the role of oxidative stress in PFOA–induced hepatic injury. TBARS is a widely used marker of oxidative damage to cell membranes, reflecting the extent of lipid peroxidation. Treatment with silymarin, naringenin and eugenol significantly reduced TBARS levels, highlighting their potent antioxidant capacities. This reduction in lipid peroxidation is consistent with previous reports demonstrating the antioxidant potential of these compounds in mitigating oxidative damage and restoring hepatic antioxidant defences (23, 24).

Histopathological evaluation revealed that PFOA exposure induced moderate microvesicular steatosis, minimal necrosis and occasional single–cell necrosis in hepatic tissues, indicating the development of hepatocellular injury. Silymarin treatment partially attenuated these changes, as expected from its known hepatoprotective properties. Notably, naringenin and eugenol, particularly at higher doses, showed substantial restoration of normal hepatic architecture, marked by the absence of steatosis and necrosis and only minimal inflammatory cell infiltration. These observations reinforce the superior efficacy of these phytochemicals in counteracting PFOA-induced histological damage. Specifically, the high-dose eugenol group exhibited the most pronounced hepatoprotection, aligning with its established roles in mitigating oxidative stress and inflammation within hepatic tissues (25, 26).

The combined antioxidant, anti–inflammatory and lipid-lowering effects of naringenin and eugenol contributed significantly to their hepatoprotective efficacy in this study. The reduction in hepatic lipid peroxidation and inflammation by these compounds highlights their potential as therapeutic agents against PFOA-induced hepatic toxicity (27, 28). To elucidate the mechanistic basis of these protective effects, an *in silico* molecular docking study was performed to examine the interactions of naringenin and eugenol with adiponectin, a regulatory protein implicated in lipid metabolism, glucose homeostasis and inflammatory processes. The PFOA exhibited weak binding affinity to adiponectin (-5.6 kcal/mol) with limited hydrophobic interactions and minimal hydrogen bonding, which is consistent with its known role in disrupting adiponectin-mediated pathways and exacerbating hepatic lipid accumulation (29).

Conversely, naringenin and eugenol displayed higher binding affinities (-6.0 and -6.5 kcal/mol, respectively), with significant hydrophobic interactions and hydrogen bonding to key active site residues of adiponectin. These interactions suggest that both compounds may stabilise the adiponectin structure or enhance its functional interactions, thereby potentially contributing to their observed hepatoprotective effects. Clinical and preclinical evidence further supports this mechanistic rationale. For example, naringin, the glycosylated form of naringenin, has been reported to increase adiponectin levels and improve lipid profiles in dyslipidemic patients (30), while naringenin has been shown to upregulate adiponectin expression and ameliorate glucose and lipid homeostasis in ovariectomised mice (31). Eugenol's strong docking affinity with adiponectin is consistent with its reported biological effects, including glucose regulation and anti–inflammatory actions, which are mediated through the activation of the adenosine monophosphate-activated protein kinase (AMPK) signalling pathway, a key regulator of adiponectin synthesis and function (32). Moreover, recent studies confirming the lipid–lowering and anti–steatotic effects of naringenin and eugenol in high–fat diet models further support their therapeutic relevance in combating PFOA–induced hepatic dysfunction (33). Collectively, these results indicate that the ability of naringenin and eugenol to interact with and modulate adiponectin or its downstream pathways may be central to their protective mechanisms against PFOA–induced hepatotoxicity. This highlights their potential as promising candidates for the prevention or management of liver damage induced by environmental toxins and warrants further investigation to explore their clinical applications and the precise molecular pathways involved.

Conclusion

The present study demonstrated the hepatoprotective potential of eugenol and naringenin against PFOA–induced hepatotoxicity in Sprague Dawley rats. Administration of PFOA resulted in marked elevations of serum transaminases, cholesterol, triglycerides and hepatic TBARS levels, along with histopathological changes indicative of hepatic steatosis, necrosis and inflammation. Treatment with eugenol and naringenin effectively ameliorated these biochemical and histopathological alterations, underscoring their antioxidant, anti-inflammatory and lipid-lowering properties. Molecular docking analyses further supported the interaction of eugenol and naringenin with adiponectin, suggesting a mechanistic role for adiponectin modulation in their hepatoprotective effects. Overall, the novelty and impact of this work lie in integrating *in vivo* biochemical and histopathological evidence with target-oriented docking to implicate adiponectin as a potential molecular link through which these phytochemicals may mitigate PFOA-induced hepatic injury. Limitations of the present findings include the short–duration exposure model and the use of a single animal species; mechanistic validation of adiponectin pathway modulation (e.g., circulating adiponectin, receptor expression and downstream AMPK signalling) was not performed and docking predictions do not substitute for experimental target engagement. Future studies should therefore incorporate molecular confirmation, pharmacokinetic/bioavailability assessment and longer-term/chronic PFAS exposure models to strengthen translational relevance.

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Authors' contributions

ST conducted the experiments, collected data and wrote the first draft of the manuscript. BJK designed the study, supervised the research, analysed the data and revised the manuscript critically for important intellectual content. AR contributed to the histopathological analysis and wrote the results of the histopathological analysis. All the authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: All experimental procedures in animals were conducted after obtaining approval from the Institutional Animal Ethics Committee (IAEC) of College of Veterinary and Animal Sciences, Mannuthy, Thrissur, India, with sanction number CVAS/MTY/IAEC/24/67. The study complies with the ARRIVE and CCSEA guidelines for animal research.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT in order to improve language and readability. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

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