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Protective effects of squalene nanoparticles against Pfoa-induced hepatotoxicity: Serum biochemical insights

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Abstract

Perfluorooctanoic acid (PFOA), a persistent environmental pollutant, induces hepatotoxicity through oxidative stress and disrupted serum biochemistry. This study evaluated the protective efficacy of squalene nanoparticles (SQ-NPs) against PFOA-induced liver toxicity in Wistar albino rats, compared to the standard antidote N-acetyl cysteine (NAC). Wistar albino rats (n = 30) were divided into five groups: control, PFOA (5 mg/kg, oral, 28 days), PFOA + NAC (50 mg/kg), PFOA + SQ (25 mg/kg), PFOA + low dose SQ-NPs (25 mg/kg) and PFOA + high dose SQ-NPs (25 mg/kg) alone. PFOA administration significantly elevated serum biochemical markers of liver injury, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH), confirming hepatic damage. Co-administration of SQ-NPs effectively normalized these levels to near control values similar to NAC, which showed partial restoration. These findings highlight squalene nanoparticles as a superior therapeutic agent against PFOA-induced hepatotoxicity, warranting further clinical exploration.

Keywords: PFOA (Perfluorooctanoic acid), SQ (squalene), NAC (N-acetyl cysteine), SQNP (squalene nanoparticles)

1. Introduction

Perfluorinated compounds (PFCs) are substances with special properties that are recognized to have many significant uses in engineering and manufacturing. Despite the fact that these materials have been used extensively over the course of the last century, concerns about the compounds potential harm to the environment have just recently emerged, and studies on exposure to humans and animals are intensifying^[1]. Concern over human exposure to perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) is growing because these chemicals are persistent in the environment, bioaccumulate and biomagnify along food chains, and have been shown to have negative health effects on development and other areas in experimental animals. PFCs are widely utilized in the production of consumer and industrial goods, such as food packaging, waterproof surfactants, lubricants, polishes, paper and clothing coatings, and firefighting foams that are used to detect fires close to airports and military installations^[2, 3]. Strong carbon-fluorine linkages make it nearly nonbiodegradable and extremely persistent in the environment^[4]. Specifically, PFOA and PFOS have been found in human tissue samples from non-occupationally exposed individuals, including the liver, kidney, adipose tissue, brain, basal ganglia, hypophysis, thyroid, gonads, pancreas, lung, skeletal muscle, and blood^[5]. According to data from NHANES 1999-2000, 2003-2004, 2005-2006, and 2007-2008, the geometric mean serum PFOA concentrations in the US population were 5.2, 3.95, 3.92, and 4.13 ng/mL, respectively^[6]. The possible adverse implications of PFOA on the health of humans and animals have drawn more attention in recent years. A range of toxicities, including hepatotoxicity^[7], carcinogenicity^[8], neurotoxicity^[9], mutagenicity^[10], developmental toxicity^[11], immunotoxicity^[12], and genotoxicity^[13], have been demonstrated by laboratory investigations to be induced by PFOA.

The main organ in the body that is harmed by PFOA poisoning is the liver. PFOA exposure has been shown in numerous studies to have harmful effects on the liver^[14-17]. Superior bioactive properties make a variety of plant terpenes useful as antimicrobials, antibiotics,

flavorings, vitamins, and repellents. Lutein, carveol, geraniol, stevioside, β -carotene, and limonene are other interesting terpenes. Squalene (SQ) is regarded as a triterpene that has both therapeutic and nutritional benefits. Squalene can act as a purifier of xenobiotic compounds in the human body because of its nonpolar nature and affinity for nonionized medicines. When SQ is included in the diet at 8% quantities, it has been discovered to enhance the removal of hexachlorobenzene (HCB, organochlorine xenobiotic) through feces. When SQ intake is higher, other xenobiotics as strychnine and theophylline can also be removed in feces [18].

Cyclophosphamide, when administered intraperitoneally caused oxidative stress and major metabolic alterations in rat tissue of the heart, as evidenced by a considerable reduction in enzymatic antioxidant activity. Squalene taken orally, however, brought these antioxidant enzyme levels back to practically normal [19]. According to other findings, a diet high in fiber, antioxidants and SQ-rich foods may be associated with a lower risk of cancer. This is the case with the Mediterranean diet, which has a low incidence of degenerative diseases due to the high consumption of SQ and phenolic compounds from fish, vegetables and fruits [20, 21].

This study evaluates the hepatotoxicity induced by perfluorooctanoic acid (PFOA) and the protective effects of squalene nanoparticles. PFOA exposure triggers oxidative stress, inflammation and cellular damage in liver tissue, leading to impaired liver function. Squalene nanoparticles counteract these effects by enhancing antioxidant defenses, reducing inflammatory markers and promoting hepatocyte recovery. Overall, the findings highlight squalene nanoparticles as a promising therapeutic agent against PFOA-induced liver injury.

2. Materials and Methods

2.1 Animal Study

Thirty Wistar albino rats of 8-12 weeks of age of either sex with average weight of 150-200 g were obtained from Small Animal Breeding Station, College of Veterinary and Animal Sciences (CVAS), Mannuthy, Kerala. In general, animal experimentation, from the beginning to the end of the study, was ethically done according to the protocol approved by Institutional Animal Ethics Committee (IAEC) as per certificate no. CVAS/MTY/IAEC/24/70 and performed in accordance with the guidelines of Committee for Control and Supervision of Experiments on Animals (CCSEA), Government of India. The rats were housed in plastic cages as three rats per cage, and maintained in the animal house facility of the Department of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy. They were acclimatised for a period of 14 days, maintained under standard conditions of temperature $25 \pm 2^\circ\text{C}$, relative humidity $70 \pm 5\%$ and a 12 h light-dark cycle. Feeding of the animals was done with standard rat pellets obtained from School of Animal Nutrition and Feed Technology, College of Veterinary and Animal Sciences, Mannuthy and for water intake tap water *ad libitum* was given throughout the experiments. Statistical analysis was done using Graph Pad Prism v.5.

2.2 Test Substances

Perfluorooctanoic acid (95%, 171468-5G) was procured from M/s Sigma-Aldrich, India. Squalene (98%, 2.05 m, S3626-10 mL) was purchased from M/s Sigma-Aldrich,

India. N-acetylcysteine (technical grade) was purchased from Cure and Care Agencies, Palakkad, Kerala. Squalene nanoparticles were synthesized and characterization was done earlier and acute oral toxicity study revealed LD50 greater than 2000 mg/kg.

Table 1: Experimental groups and administration of test substance

Group	Treatment
I	PFOA @ 5 mg/kg in 0.4% DMSO
II	PFOA @ 5 mg/kg + N-acetyl cysteine @ 50 mg/kg
III	PFOA @ 5 mg/kg + Squalene @ 25 mg/kg
IV	PFOA @ 5 mg/kg + low dose squalene loaded PLGA nanoparticles (25 mg/kg)
V	PFOA @ 5 mg/kg + high dose squalene loaded PLGA nanoparticles (25 mg/kg)

Hepatic function was assessed every week by estimating the alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in serum using standard kits (Yang *et al.*, 2014). All the kits used for serum analysis were procured from M/s Agappe Diagnostics Ltd., India.

3. Results

3.1 Alanine amino transferase (ALT)

The concentration of ALT in the serum of the experimental animals is shown in table 2. During the first and second week of the study there was no significant difference in ALT values among different groups. In the latter half of the study group I (83.77 ± 6.49) varied significantly from other groups, as evident in fig 1. The group I (87.29 ± 5.89) showed highest increase of ALT levels in serum.

3.2 Aspartate amino transferase (AST)

The concentration of AST in the serum of the experimental animals is shown in table 3. During the first and second week of the study there was no significant difference in AST values among different groups. In the third week group II (102.73 ± 4.73) varied significantly from other groups, whereas in fourth week there was significant difference among different groups as evident in fig 2. The group I (161.58 ± 2.55) showed highest increase of AST levels in serum.

3.3 Alkaline phosphatase (ALP)

The concentration of ALP in the serum of the experimental animals is shown in table 4. During the first and second week of the study there was no significant difference in ALP values among different groups. In the third week there was no significant difference between group II and IV but group I (725.99 ± 31.74) varied significantly among other groups, whereas in fourth week there was significant difference between group I (704.07 ± 12.61) and other groups but there was no significant difference between group II (416.25 ± 23.23) group III (597.85 ± 21.71) and IV (497.23 ± 21.36), as evident in fig 3. The group I (704.07 ± 12.61) showed highest increase of ALP (704.07 ± 12.61) levels in serum.

3.4 Lactate dehydrogenase (LDH)

The concentration of LDH in the serum of the experimental animals is shown in table 5. During the first and second week of the study there was no significant difference in LDH values among different groups. In the third week

significant and group I (781.29 ± 35.39) varied significantly from other groups, whereas in fourth week there was significant difference between group I (842.53 ± 27.34) and

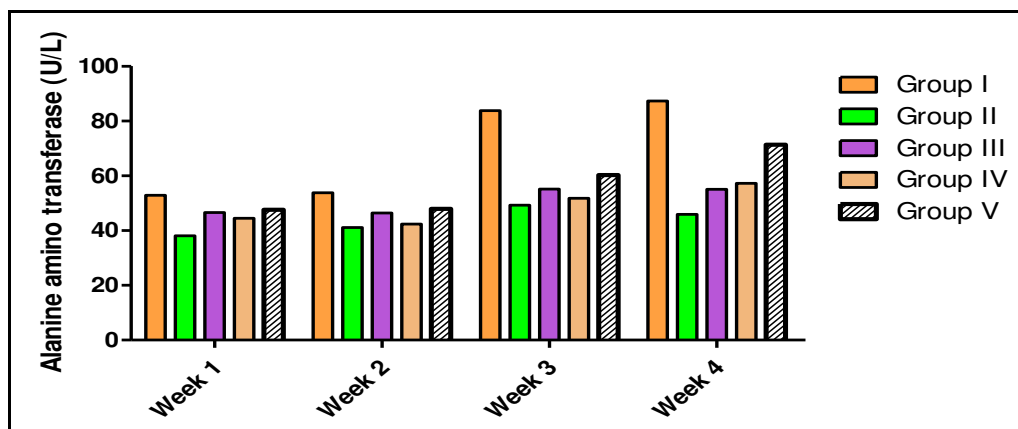
groups II (610.79 ± 22.6), IV (606.89 ± 25.38) as evident in fig 4. The group I (842.53 ± 27.34) showed highest increase of LDH levels in serum.

Table 2: Mean weekly serum ALT (U/L) levels of different groups

Groups	Week 1	Week 2	Week 3	Week 4
I (PFOA)	52.87 ± 3.54	53.78 ± 2.62	$83.77^b \pm 6.49$	$87.29^b \pm 5.89$
II (PFOA + NAC)	38.05 ± 3.91	41.14 ± 2.61	$49.27^a \pm 3.91$	$45.91^a \pm 2.04$
III (PFOA + SQ)	46.54 ± 3.05	46.35 ± 3.3	$55.14^a \pm 5.54$	$55.03^a \pm 3.7$
IV (PFOA + Low dose SQ-NP)	44.45 ± 3.6	42.34 ± 4.24	$51.76^a \pm 5.4$	$57.18^a \pm 3.85$
V (PFOA + High dose SQ-NP)	47.56 ± 3.4	47.88 ± 3.25	$60.26^a \pm 3.99$	$71.36^b \pm 3.78$

Values are expressed as Mean $\pm$ SEM, n = 6

Values bearing different superscripts vary significantly at $p < 0.05$ in the column PFOA-perfluorooctanoic acid; NAC-N-acetylcysteine; SQ-squalene; SQ-NP-Squalene loaded PLGA nanoparticles



Group I-PFOA; Group II-PFOA + NAC; Group III-PFOA + SQ; Group IV-PFOA + low dose SQ-NP; Group V-PFOA + high dose SQ-NP
PFOA-perfluorooctanoic acid; NAC-N-acetylcysteine; SQ-squalene; SQ-NP-squalene loaded PLGA nanoparticles.

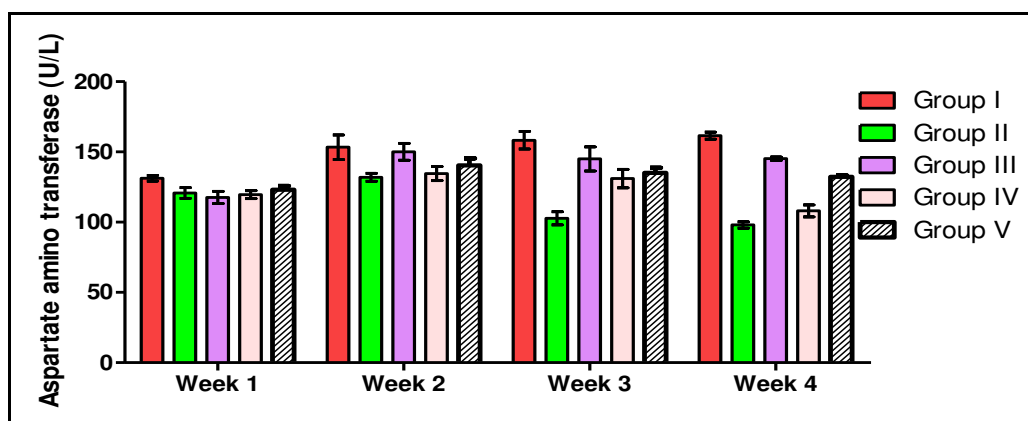
Fig 1: Mean weekly serum ALT levels of different groups

Table 3: Mean weekly serum AST (U/L) levels of different groups

Groups	Week 1	Week 2	Week 3	Week 4
I (PFOA)	131.24 ± 1.99	153.36 ± 8.7	$158.3^b \pm 6.21$	$161.58^d \pm 2.55$
II (PFOA + NAC)	120.74 ± 3.82	131.91 ± 2.81	$102.73^a \pm 4.73$	$98.05^a \pm 2.22$
III (PFOA + SQ)	117.61 ± 4.36	150.14 ± 6.54	$145.79^b \pm 8.57$	$145.19^c \pm 1.23$
IV (PFOA + Low dose SQ-NP)	119.55 ± 2.8	134.64 ± 5.28	$131.2^b \pm 6.58$	$108.04^a \pm 4.29$
V (PFOA + High dose SQ-NP)	123.06 ± 2.76	140.38 ± 5.33	$135.84^b \pm 3.76$	$132.25^b \pm 0.93$

Values are expressed as Mean $\pm$ SEM, n = 6

Values bearing different superscripts vary significantly at $p < 0.05$ in the column PFOA-perfluorooctanoic acid; NAC-N-acetylcysteine; SQ-squalene; SQ-NP-Squalene loaded PLGA nanoparticles



Group I-PFOA; Group II-PFOA + NAC; Group III-PFOA + SQ; Group IV-PFOA + low dose SQ-NP; Group V-PFOA + high dose SQ-NP
PFOA-perfluorooctanoic acid; NAC-N-acetylcysteine; SQ-squalene; SQ-NP-squalene loaded PLGA nanoparticles

Fig 2: Mean weekly serum AST levels of different groups

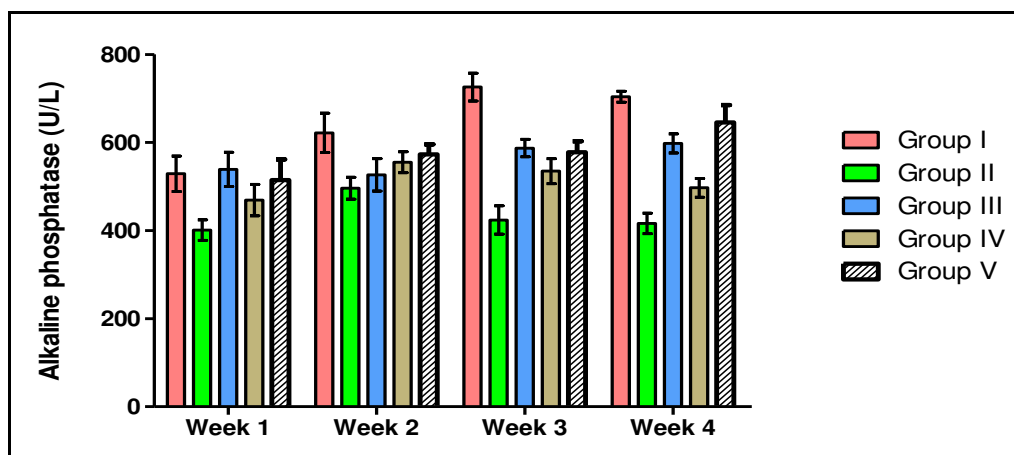
Table 4: Mean weekly serum ALP (U/L) levels of different groups

Groups	Week 1	Week 2	Week 3	Week 4
I (PFOA)	529.02±40.29	621.96±44.76	725.99 ^a ±31.74	704.07 ^a ±12.61
II (PFOA + NAC)	401.07±23.36	496.54±22.71	423.99 ^b ±32	416.25 ^b ±23.23
III (PFOA + SQ)	539.03±38.79	526.52±37.96	587.37 ^b ±19.8	597.85 ^b ±21.71
IV (PFOA + Low dose SQ-NP)	469.17±35.89	555.23±24.85	535.75 ^b ±28.39	497.23 ^b ±21.36
V (PFOA + High dose SQ-NP)	514.41±47.31	572.84±23.35	578.98 ^b ±24.62	645.33 ^a ±39.67

Values are expressed as Mean ± SEM, n = 6

Values bearing different superscripts vary significantly at $p < 0.05$ in the column

PFOA-perfluorooctanoic acid; NAC-N-acetylcysteine; SQ-squalene; SQ-NP-Squalene loaded PLGA nanoparticles



Group I-PFOA; Group II-PFOA + NAC; Group III-PFOA + SQ; Group IV-PFOA + low dose SQ-NP; Group V-PFOA + high dose SQ-NP

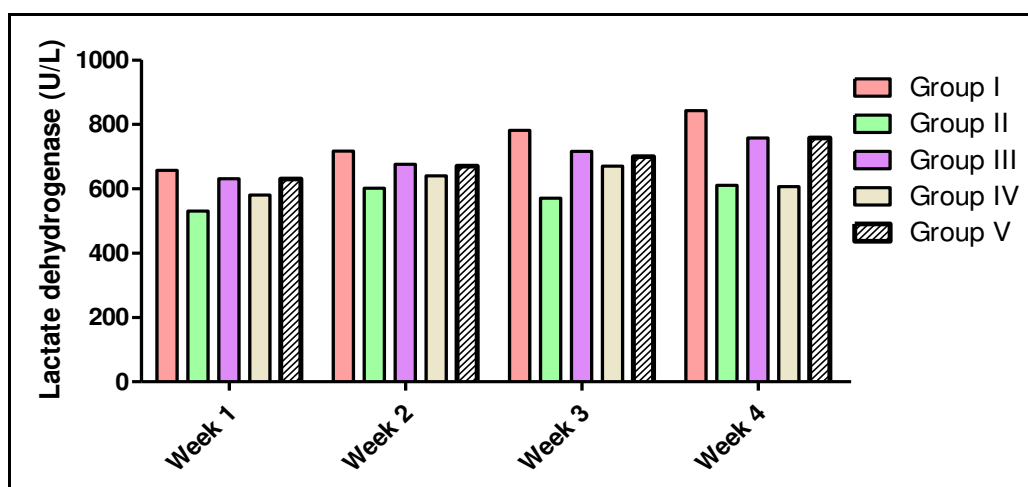
PFOA-perfluorooctanoic acid; NAC-N-acetylcysteine; SQ-squalene; SQ-NP-squalene loaded PLGA nanoparticles

Fig 3: Mean weekly serum ALP levels of different groups**Table 5:** Mean weekly serum LDH levels (U/L) of different groups

Groups	Week 1	Week 2	Week 3	Week 4
I (PFOA)	657.33±34.47	716.6±15.05	781.29 ^b ±35.39	842.53 ^b ±27.34
II (PFOA + NAC)	530.45±20.72	601.42±42.3	570.66 ^a ±42.44	610.79 ^a ±22.6
III (PFOA + SQ)	630.84±35.76	676.78±21.68	715.79 ^a ±16.85	757.43 ^b ±31.8
IV (PFOA + Low dose SQ-NP)	580.4±39.27	640.75±32.62	670.51 ^a ±44.08	606.89 ^a ±25.38
V (PFOA + High dose SQ-NP)	629.97±47.68	670.6±21.4	700.09 ^a ±26.01	757.58 ^b ±28.91

Values are expressed as Mean ± SEM, n = 6

Values bearing different superscripts vary significantly at $p < 0.05$ in the column PFOA-perfluorooctanoic acid; NAC-N-acetylcysteine; SQ-squalene; SQ-NP-Squalene loaded PLGA nanoparticles



Group I-PFOA; Group II-PFOA + NAC; Group III-PFOA + SQ; Group IV-PFOA + low dose SQ-NP; Group V-PFOA + high dose SQ-NP

PFOA-perfluorooctanoic acid; NAC-N-acetylcysteine; SQ-squalene; SQ-NP-squalene loaded PLGA nanoparticles

Fig 4: Mean weekly serum LDH levels of different groups

Discussion

Environmental contaminants have been shown to have negative impacts on both human and animal health. Many industrial operations use PFOA, a persistent chemical that has been detected in both general populations and individuals who have been exposed to it at work [21]. The liver exhibits particular susceptibility to injury following systemic exposure to xenobiotics, owing to its pivotal role in drug metabolism and detoxification processes [22]. Studies have reported a positive association between PFOA exposure and hepatic injury in humans [23]. Animal studies have likewise demonstrated the hepatotoxic effects of PFOA [14-17]. Serum transaminases represent the most universally accepted indicators for diagnosing hepatic injury. Hepatocellular damage compromises the integrity of the plasma membrane, permitting the release of intracellular enzymes such as ALT and AST into the systemic circulation. This leakage consequently results in elevated serum transaminase levels, serving as a sensitive biomarker of liver dysfunction [24]. LDH serves as another key leakage enzyme and potential marker of hepatocellular toxicity. ALP is commonly used to assess impaired bile flow, with elevated serum levels primarily linked to hepatobiliary origins. In this study, oral PFOA administration significantly increased serum AST, ALT, ALP, and LDH levels. Notably, Group I rats treated solely with PFOA exhibited marked elevations in these markers [25].

Numerous studies have demonstrated the effectiveness of N-acetylcysteine (NAC) in alleviating toxicity driven by oxidative mechanisms [26, 27, 28]. The thiol moiety of NAC is highly bioavailable, relatively stable and safe, while also displaying potent antioxidant properties [29, 30]. In this study, Group II rats treated with NAC exhibited reduced serum enzyme levels [25]. Group III rats administered squalene (SQ) successfully reduced serum enzyme levels [31]. However, the elevated levels of serum hepatic enzymes were significantly decreased by simultaneous treatment with squalene nanoparticles. The findings of present study indicated that low dose SQ-NP exhibited significant therapeutic potential in protecting liver function during PFOA induced hepatotoxicity. The antioxidant and anti-inflammatory properties of squalene likely played a crucial role in mitigating oxidative stress and cellular damage associated with PFOA exposure, thereby preserving liver integrity. Additionally, the ability of SQ-NP to restore liver enzyme levels to normal ranges, comparable to those achieved with NAC, culminated its protective effect against hepatotoxicity. This accentuated the promise of squalene nanoparticles as an effective intervention for maintaining hepatic health in the face of toxic environmental exposures.

Conflicts of Interest

The authors declare that there are no conflicts of interest

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