Research Article

The Protective Role of Black Seed Oil on Perfluorooctanoic Acid -Induced Growth, Organ Dysfunction and Hepato-Renal Toxicity in Swiss Albino Female Mice

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Abstract | Perfluorooctanoic acid (PFOA), an industry-derived obstinate environmental chemical, bioaccumulates in the liver and kidneys, severely disrupting detoxification processes by inducing oxidative stress, inflammation, lipid accumulation, fibrosis, and cellular apoptosis. This study investigated the effects of black seed oil (BSO), a powerful bioactive compound derived from *Nigella sativa* (black seed), on PFOA-induced hepato-renal toxicities in Swiss albino mice. Eighteen female albino mice of 28-30 days of age were used and divided into three groups (n=6). Group A (control) was designated as the non-treated control group and fed normal mice pellets. Mice of group B were fed a PFOA mixture, while group C received a PFOA mixture and BSO for 60 days. Body weights were recorded at 15-day intervals. Blood and selected organs were collected and processed for liver and kidney function tests and histopathological study. PFOA exposure significantly decreased body weight in female mice in a time-dependent manner and increased absolute and relative liver weights causing hepatomegaly. There was a marked elevation in liver enzymes ALT and AST levels, indicating hepatic injury. Serum creatinine was also elevated, suggesting impaired renal function. Histological observations revealed hepatocyte enlargement, fatty changes, and renal cellular damage including as pyknotic nuclei. Co-treatment with BSO significantly reduced liver enzyme levels and improved the histoarchitecture of the liver and kidneys by mitigating fatty infiltration and ameliorating renal changes, although some abnormalities persisted in the kidney. These findings denote the potential of BSO as a promising therapeutic agent in minimizing liver damage and partly kidney damage caused by PFOA exposure.

Keywords | Black seed oil (BSO), Mice, Perfluorooctanoic acid, Liver enzymes, Histopathology

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INTRODUCTION

Per- and Polyfluoroalkyl Substances (PFAS) comprise a group of over 9,000 synthetic chemicals widely used in industrial and consumer applications. These include the manufacture of non-stick cookwares, water-resistant fabrics, and firefighting foam carpets, textiles, cable and wire coatings, food packaging, and personal care products (Baker and Knappe, 2022). Perfluorooctane sulfonate (PFOS) and Perfluorooctanoic acid (PFOA) are well studied representatives of the PFAS group (Tsuda, 2016). They are highly stable and resist chemical, biological, and

physical breakdown, leading to their extreme persistence in the environment a trait that has earned them the nickname "forever chemicals" (De Silva *et al*., 2021; Baker and Knappe, 2022). In particular, PFOA is a significant environmental pollutant within the perfluoroalkyl group. Due to its high-water solubility and stability, it does not degrade easily, resulting in widespread detection in air, soil, groundwater, wildlife, and human tissues (Epa, 2012; Liu *et al*., 2023). This pervasive presence has raised substantial concern regarding its potential adverse effects on ecosystems and human health. Due to its environmental persistence, bioaccumulation potential, and long biological half-life, PFOA has become a global health concern, with widespread human exposure occurring through contaminated water, food, and consumer products (Wang *et al*., 2017; Li *et al*., 2018). Furthermore, infants are exposed via placental transfer and breast milk, potentially leading to adverse health effects as they grow (Awad *et al*., 2020). Workers, interior decorators primarily exposed via inhalation and dermal contact with contaminated dust, respectively (Franko *et al*., 2012; Sunderland *et al*., 2019). Epidemiological and experimental studies have linked PFOA exposure to multiple adverse health effects, including hepatotoxicity, nephrotoxicity, endocrine disruption, immune suppression, and developmental abnormalities (Sunderland *et al*., 2019). The liver and kidneys are particularly vulnerable, as PFOA accumulates in these organs, inducing oxidative stress, inflammation, and cellular damage (Zahm *et al*., 2024). PFOA is carcinogenic resulting in liver, kidney, and testicular cancers in humans. Moreover, it contributes to breast cancer by promoting the growth and invasion of breast epithelial cells, a mechanism mediated by PPARα pathways (Pierozan *et al*., 2018). PFOA exposure is also associated with non-cancer health effects including pneumonia, weight loss, and abnormal behavior (Cui *et al*., 2009; Buhrke *et al*., 2015).

Nigella sativa, commonly known as black seed or black cumin, is a member of Ranunculaceae family, and has been used as a potent medicinal herb for many centuries (Razmpoosh *et al*., 2020). The nutritional and medicinal value comes from a wealth of bioactive components, including linoleic and oleic acids, vital minerals (calcium, magnesium, iron) and potent antioxidants from phenolic and flavonoid compounds (Razmpoosh *et al*., 2020; Hossain *et al*., 2024). The identified key therapeutic agents of *N. sativa* are Thymoquinone, ß-Pinene, O-Cymene, Terpinen-4-ol, Limonen-6-ol, Longifolene, and Stigmasterol. The major active constituent thymoquinone exhibits strong antioxidant, anticancer, antiproliferative, immunomodulatory, and antibacterial properties (Majdalawieh *et al*., 2017; Tiji *et al*., 2021; Alsalahi *et al*., 2024; Abbas *et al*., 2024). Research indicates that thymoquinone provides a hepatoprotective effect by guarding the liver against oxidative damage (Mollazadeh and Hosseinzadeh, 2014). This is achieved through neutralizing free radicals, boosting the body's antioxidant mechanisms, and inhibiting inflammatory responses (Mollazadeh and Hosseinzadeh, 2014). Similarly, *Nigella sativa* offers renal protection by mitigating oxidative stress, preventing DNA damage, and reducing tissue lesions in the kidneys (Havakhah *et al*., 2014). It also demonstrates a beneficial effect on organ functions by reducing the elevated levels of biomarkers such as ALP, BUN, and AST (Razmpoosh *et al*., 2020). Therefore, this study investigated the protective effects of Black Seed Oil (BSO) against PFOA-induced liver and kidney damage in Swiss albino mice by examining physiological, biochemical, and histopathological changes.

MATERIALS AND METHODS

Experimental animals and study plan

Eighteen (18) female Swiss Albino mice (*Mus musculus*), aged 28-30 days, were used in this study. The mice were reared in polypropylene cages housed in a temperature-controlled, well-ventilated room. The room temperature was maintained at 28 ± 2°C, with relative humidity ranging from 70-80%, and natural daylight was used as the light source. Strict hygienic and sanitary practices were followed throughout the experimental period. During a 15-day acclimatization period, all mice were provided with standard mice pellets purchased from ICDDR'B, Dhaka and clean drinking water *ad libitum*. On the 16th day, their average body weight was recorded, and the mice were randomly divided into three groups (A, B, and C), each containing six (6) mice. Group A served as the vehicle control and was fed a normal pellet diet, Group B received Perfluorooctanoic acid (PFOA) (Sigma-Aldrich Company, Germany) at 30 mg/kg/day via drinking water, and group C was administered PFOA (30 mg/kg/day) with BSO obtained from a local market, (2 ml/kg/day) mixed in their feed. Body weight, behavioral patterns, and clinico-pathological changes were observed regularly throughout the experimental periods that lasted 60 days.

Body weight, organ weight, and relative organ weight measurements

The body weight of each mouse was recorded using a digital balance at the onset of the treatment and subsequently at 15-day intervals until the end of the experimental period (Sarker *et al*., 2019). Upon dissection, the liver, kidney, and spleen were carefully isolated, ensuring the removal of all adhering connective tissues, and each organ was weighed individually with precision. To assess the relative organ weight, the weight of each organ was divided by the live body weight of the respective mice and multiplied by 100, providing the organ weight as a percentage of the total body weight. This method allowed for a standardized

evaluation of organ weight changes relative to overall body mass (Hidayat *et al*., 2017).

Blood collection and serum biochemical studies

Blood samples were collected from the mice of each group on the final day of the experiment (day 60), according to the standard published protocol (Sarker *et al*., 2019). Sera was collected and kept in the refrigerator at -20 ˚C for biochemical analysis. The biochemical parameters of serum AST, ALT, creatinine, and uric acid were performed at Professor Dr. Mohammad Hussain Central Laboratory in Bangladesh Agricultural University, Mymensingh-2200 using a UV spectrophotometer T 80, PG instruments, Great Britain. Specific reagents from High Technology Incorporation (HTI, USA) were used for each test (Rakib *et al*., 2021).

Histopathology of liver and kidney tissues

The livers and kidneys of each group of mice were collected after the blood was completely removed by perfusion with phosphate-buffered saline and stored in 10% neutral buffered formalin for 15 days. The well-fixed tissues were subjected to standard paraffin-embedding procedures: dehydration through graded alcohols, clearing in xylene, infiltration with molten paraffin wax, and embedding in paraffin blocks. Sections of 4–5 µm thickness were cut using a rotary microtome, mounted on glass slides, and stained with Hematoxylin and Eosin (H&E) following the established protocol described by (Suvarna *et al*., 2018). The stained sections were examined under a light microscope to assess histopathological alterations.

Statistical analysis

Data was analyzed using GraphPad Prism 8 software specifically employing a one-way Analysis of Variance (ANOVA) with Post-hoc Tukey's test. Significance was considered at $p < 0.05$ or $p < 0.01$ (Miah *et al*., 2022).

RESULTS

Effect of PFOA and BSO on body weight gain in Swiss albino mice

The average weight gain in mice treated with PFOA and PFOA combined with BSO is presented in (Figure 1). No clinical signs and mortality were observed throughout the experimental periods. However, PFOA caused a significant, time-dependent reduction in body weight gain in mice compared to the control group ($P<0.01$). Furthermore, concurrent treatment with BSO partially mitigated this weight loss, with the PFOA+BSO group (27.0 ± 0.50 g) achieving a higher weight than the PFOA treated group (24.0 ± 0.50 g), which was significantly lower than the control (40.0 ± 0.51 g). Body weight was significantly reduced in the PFOA group compared with the Control group ($P < 0.01$), whereas supplementation with BSO significantly improved body weight compared to the PFOA group ($P < 0.01$).

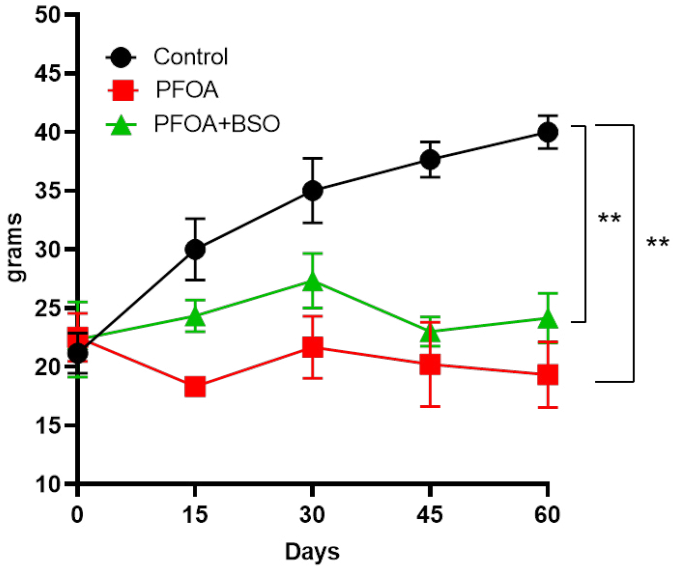

Figure 1: Effect of BSO on body weight in PFOA treated female mice in a time dependent manner. **P<0.01 (control versus PFOA; control versus PFOA+BSO on day 30, 45 and 60 of ages).

Effect of PFOA and BSO on weights, gross morphology and pathology of liver and kidney in Swiss albino mice

On gross morphological observations, the liver of PFOA treated mice were markedly enlarged, congested and discolored. However, the mice co-treated with PFOA+BSO showed normal liver morphology with mild congestion, though the organ remained somewhat enlarged compared to control (Figure 2A).

Kidneys appeared smaller in PFOA-treated mice, with partial improvement in the PFOA + BSO group (Figure 2A). These results indicate that PFOA significantly altered organ weights and morphology, while BSO exhibited a protective effect by partially normalizing these changes.

The absolute liver weight was highest in PFOA treated mice (Group B) at 6.29 ± 1.10 g, followed by PFOA + BSO treated group (Group C) at 4.75 ± 1.76 g, and lowest in control group (Group A) at 2.76 ± 0.25 g (Figure 2B) Similarly, the relative liver weight was significantly increased in the PFOA-treated group (29.92 ± 4.51%) compared to the control (5.74 ± 0.67%) and PFOA + BSO (18.50 ± 5.10%) groups, with statistical significance ($P<0.01$), indicating significant liver enlargement and absolute increase in liver weight (Figure 2C).

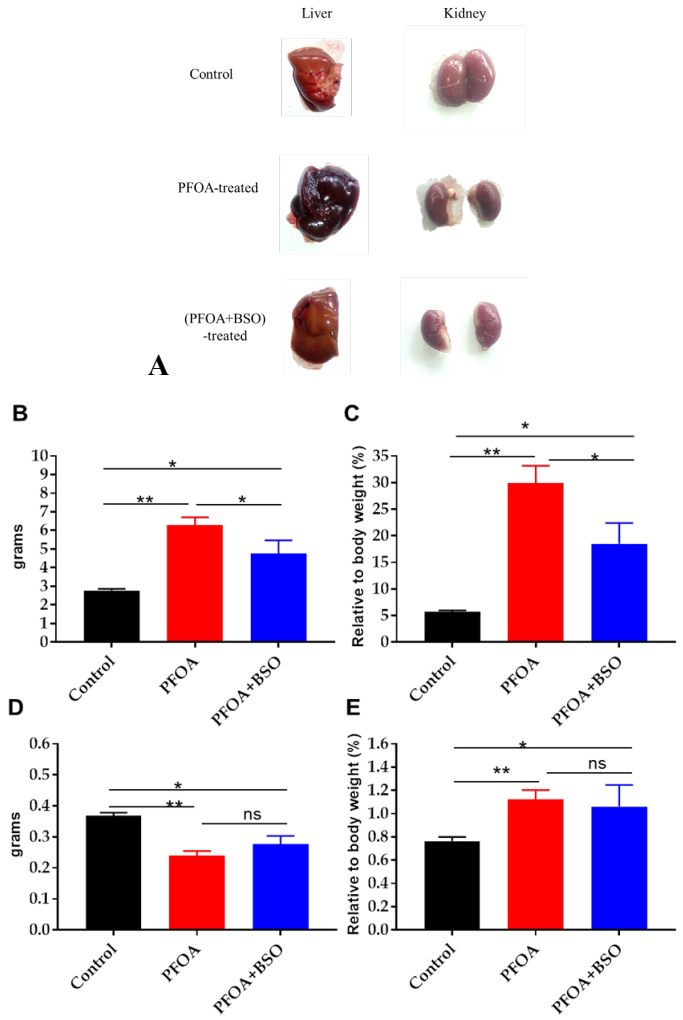

Figure 2: A: Effect of PFOA and BSO on liver and kidney gross morphology in female albino mice. It shows photomicrographs of liver, and kidney. B-E: Effect of PFOA and BSO on absolute weight and relative weights of liver and kidneys of different group of mice. ** P<0.01 (control versus PFOA); * P<0.05 (control versus PFOA+BSO) in each parameters; Ns-not significant.

The pattern of kidney weight changes differed from that of the liver. Mice from control group (Group A) had increased absolute kidney weight (0.36 ± 0.05 g), which was higher than that of PFOA + BSO (Group C) (0.30 ± 0.06 g), and PFOA treated group (Group B) (0.24 ± 0.03 g). However, when expressed as a percentage of body weight (relative weight) to account for the overall reduction in body mass, the order was inverted. The PFOA group (B) showed the highest relative kidney weight (1.12 ± 0.21%), followed by the PFOA+BSO group (C) and then the control group (A) with the lowest (0.76 ± 0.09%) (Figure 2D, E).

On histological examination, PFOA-treated mice exhibited marked fatty change characterized numerous vacuoles within the hepatocytes along with pyknotic nuclei. However, PFOA+BSO-treated mice showed reduced fatty changes, although hepatocyte enlargement persisted (Figure 3). Furthermore, kidneys from PFOA-treated and PFOA+BSO-treated mice exhibited slightly enlarged renal structures and pyknotic nuclei, indicating mild cellular injury (Data not shown).

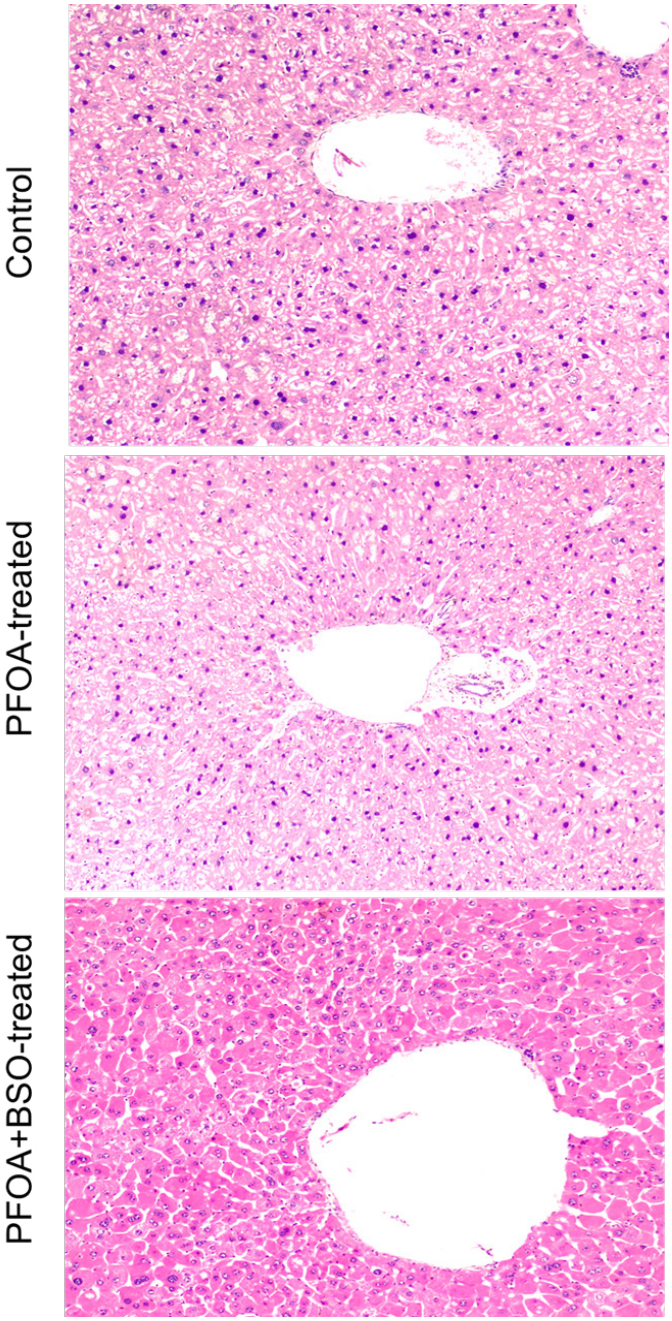

Figure 3: Histopathological changes in liver tissues of different groups of mice treated with PFOA and BSO at 60th day of experiment. Photomicrographs were taken at 100X magnification.

Effects on serum AST, ALT, creatinine and uric acid concentration

In this study, the effects of PFOA and BSO on liver and kidney functions in female albino mice were assessed through measurement of serum ALT, AST, creatinine, and uric acid levels (Figure 4). PFOA treated mice exhibited significantly increased ALT (732.22 ± 58.50 U/L) and

AST (595.09 ± 102.51 U/L) levels compared to the control group (46.78 ± 11.20 U/L and 74.43 ± 13.81 U/L, respectively), indicating liver damage (P<0.01). Mice treated with PFOA+BSO showed lower levels of the mentioned enzymes (445.63 ± 104.50 U/L for ALT and 276.45 ± 74.92 U/L for AST) but remained higher than the control (P<0.01). PFOA treatment also resulted in a significant increase in creatinine (0.65 ± 0.04 mg/dL; P<0.05), but the absolute value may still fall within a broad clinical normal range, while BSO slightly reduced creatinine levels (0.60 ± 0.06 mg/dL). Uric acid levels were slightly elevated in both PFOA (6.47 ± 0.62 mg/dL) and PFOA+BSO groups (6.55 ± 1.12 mg/dL), but no significant differences were observed among them.

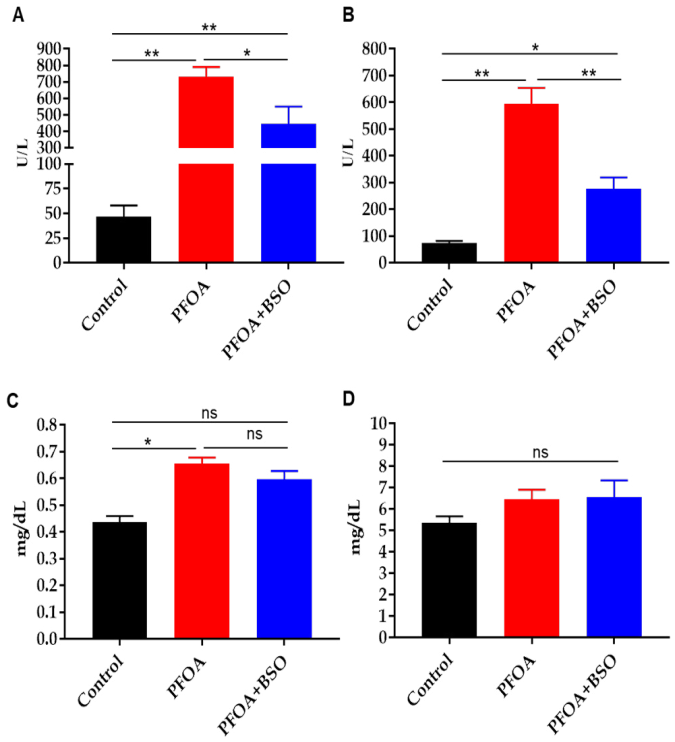

Figure 4: Effect of BSO on (A) alanine aminotransferase (ALT), (B) aspartate aminotransferase (AST), creatinine and uric acid levels in PFOA treated mice. ** P<0.01 (control versus PFOA; Control versus PFOA+BSO) * P<0.05 (PFOA versus PFOA+BSO). Ns- not significant.

DISCUSSION

The current study focused on the effects of PFOA alone and PFOA along with BSO in liver and kidneys, the major organs responsible for detoxifying and excreting foreign chemicals. PFOA exposure caused morphological damage to the liver evident as hepatomegaly, and impaired physiological function, indicated by elevated AST and ALT levels. The PFOA treated mice showed mild kidney damage with elevated creatinine and uric acid levels. However, treatment with BSO produced significant protective effects, particularly in restoring liver health.

The weight loss observed in PFOA-treated mice aligns with earlier studies, which attribute the reduction to appetite suppression and metabolic dysfunction (Attema *et al.*, 2022). In this study, partial weight recovery was observed in PFOA+BSO co-treated mice which is linked to the reduction of PFOA's general toxicity and systemic stress (Attema *et al.*, 2022). Furthermore, Black seed oil (BSO) supplementation has been shown to reduce body weight via anti-obesity mechanisms (Abo El-Magd *et al.*, 2021; Naghsh *et al.*, 2023).

Liver cells exposed to high PFOA concentrations could result in cellular injury, liver dysfunction, and eventually organ failure (Wen *et al.*, 2020). In this study, we observed significant increase in liver mass due to vacuolation and lipid accumulation within the hepatocytes resulting hepatomegaly in PFOA-exposed mice, are in line with a previous study conducted by Wen *et al.* (2020). However, mice received BSO exhibited improved liver histology, as evident by reduced lipid accumulation or vacuolation, and thus, reduction in liver weight. This finding is also consistent with the study conducted by Bashir *et al.* (2023) where Black cumin seed extract inhibited the high fat diet induced effects in liver, such as increased absolute liver weight, hepatic lipid accumulation and hepatomegaly (Bashir *et al.*, 2023). ALT and AST are the most common biochemical tests for assessing liver function or liver damage. In our study, we have found elevated levels of ALT and AST in PFOA treated mice which align with the previous work by Wu *et al.* (2017). These elevations are related to hepatocytes impairment in the PFOA-treated mice, likely resulting from enhanced expression of CD36 (fatty acid translocase) in hepatocytes and impaired hormonal pathways in pancreas (Wu *et al.*, 2017). In the current study, the elevated levels of ALT and AST in concurrently administered PFOA and BSO mice revealed the positive effects of BSO in liver toxicity over PFOA. Similar findings were also observed prior studies where BSO reduced the liver biomarker's concentration in serum due to its hepatoprotective characteristics (Rahim *et al.*, 2023; Bashir *et al.*, 2023).

PFOA accumulates in the kidneys due to its strong binding to proteins and slow renal elimination, making it as a primary target organ for toxicity (Liu *et al.*, 2023). While the current study revealed reduced kidney weight with normal histology in PFOA exposed mice, other studies frequently observed kidney hypertrophy probably due to thickening of glomerular capsule and basement membrane (Story, 2025; Wang *et al.*, 2024). In contrast, earlier studies revealed PFOA induced kidney damage due to oxidative stress, inflammation, and disruption of signaling pathways such as the peroxisome proliferator-activated receptor alpha (PPARα) pathway (Liu *et al.*, 2023). All these events actually contribute to lipid peroxidation, DNA damage,

and alterations in renal tubular functions (Liu *et al*., 2023). Furthermore, the increased levels of kidney biomarkers like urea, creatinine, and uric acid indicate kidney dysfunction. Here, PFOA treatment has increased the serum creatinine and uric acid at a significant level similar to the study reported by Conway *et al*. (2018), suggesting chronic PFOA exposure caused decreased estimated glomerular filtration rate (eGFR) and elevated albumin/creatinine ratios (Conway *et al*., 2018). Moreover, BSO therapy restored the hepatorenal histopathology and reduced kidney biomarkers levels probably due to decreased lipid peroxidation. BSO therapy induces protective response in liver and kidney is by elevating active glutathione level and increases antioxidant enzymes (Badr *et al*., 2020).

CONCLUSION

The findings of the study demonstrate that perfluorooctanoic acid (PFOA) exposure adversely affects body weight, organ size and dysfunction, and hepato-renal toxicity in Swiss albino female mice. Critically, this research establishes that co-treatment with Black Seed Oil (BSO) confers a significant protective effect against PFOA-induced toxicity. BSO effectively alleviates the morphological and functional damage in the liver and kidneys, as confirmed by the restoration of biomarker levels and the improvement of tissue architecture. This study was conducted only in female mice under controlled laboratory conditions, which limits how the generalizability of the findings. Future research should explore the underlying mechanisms, determine the most effective dose, assess long-term safety, and confirm these protective effects in both sexes and under varied exposure scenarios to strengthen their relevance for real-world applications.

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NOVELTY STATEMENT

This study represents the first investigation to evaluate the protective efficacy of black seed oil (BSO) against perfluorooctanoic acid (PFOA)-induced hepato-renal toxicity in mouse model. While the hepatoprotective and nephroprotective properties of BSO have been documented independently, and PFOA toxicity has been extensively studied, the concurrent therapeutic application of BSO to mitigate PFOA-induced organ damage has not been previously explored. This study establishes a novel therapeutic approach by demonstrating that BSO co-treatment can significantly ameliorate PFOA-induced hepatomegaly, liver enzyme elevation, and histopathological alterations in liver tissues, providing new insights into potential natural interventions against emerging environmental pollutant toxicity.

AUTHOR'S CONTRIBUTION

MAM: Designed the experiment, supervised it, analyzed data, and revised the final draft of the manuscript.
TS, SR, MKHB, SH: Carried out the experiment, analyzed data, and wrote the first draft of the manuscript.
JAB: Performed the histology of the mouse liver and kidney and revised the manuscript.
AM: Critically revised the manuscript.

Data availability statement

Data supporting the results of this study can be obtained from the corresponding author upon reasonable request.

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Ethical approval

The Animal Welfare and Experimentation Ethics Committee at Bangladesh Agricultural University, Mymensingh, granted approval for the animal care, management, and experimental procedures under reference number AWEEC/BAU/2022 (24).

Generative AI and AI-assisted technology statement

The authors used ChatGPT in order to correct grammatical errors and make sentences flow. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abbas M, Gururani MA, Ali A, Bajwa S, Hassan R, Batool SW, Imam M, Wei D (2024). Antimicrobial properties and therapeutic potential of bioactive compounds in *Nigella sativa*: A review. Molecules, 29. https://doi.org/10.3390/molecules29204914

Abo El-Magd NF, El-Mesery M, El-Karef A, El-Shishtawy MM (2021). Amelioration effect of black seed oil against high-fat diet-induced obesity in rats through Nrf2/HO-1 pathway. J. Food Biochem., 45: 1–12. https://doi.

org/10.1111/jfbc.13693

Alsalahi A, Maarof NNN, Alshawsh MA, Aljaberi MA, Qasem MA, Mahuob A, Badroon NA, Mussa EAM, Hamat RA, Abdallah AM (2024). Immune stimulatory effect of Nigella sativa in healthy animal models: A systematic review and meta-analysis. Heliyon, 10: e27390. https://doi.org/10.1016/j.heliyon.2024.e27390

Attema B, Janssen AWF, Rijkers D, van Schothorst EM, Hooiveld GJEJ, Kersten S (2022). Exposure to low-dose perfluorooctanoic acid promotes hepatic steatosis and disrupts the hepatic transcriptome in mice. Mol. Metab., 66: 101602. https://doi.org/10.1016/j.molmet.2022.101602

Awad R, Zhou Y, Nyberg E, Namazkar S, Yongning W, Xiao Q, Sun Y, Zhu Z, Bergman Å, Benskin JP (2020). Emerging per- And polyfluoroalkyl substances (PFAS) in human milk from Sweden and China. Environ. Sci. Process. Impacts, 22: 2023–2030. https://doi.org/10.1039/D0EM00077A

Badr K, Abdel Fattah A, Gaballah I (2020). Possible Protective potential of atorvastatin and black seed (*Nigella sativa*) oil in amikacin induced nephrotoxicity in adult male albino rats. Egypt. J. Forensic Sci. Appl. Toxicol., 20: 55–65. https://doi.org/10.21608/ejfsat.2020.24770.1129

Baker ES, Knappe DRU (2022). Per-and polyfluoroalkyl substances (PFAS) contaminants of emerging concern. Anal. Bioanal. Chem., 414: 1187–1188. https://doi.org/10.1007/s00216-021-03811-9

Bashir KMI, Kim JW, Kim JK, Chun YS, Choi JS, Ku SK (2023). Efficacy confirmation test of black cumin (*Nigella sativa* L.) seeds extract using a high-fat diet mouse model. Metabolites, 13. https://doi.org/10.3390/metabo13040501

Buhrke T, Krüger E, Pevny S, Rößler M, Bitter K, Lampen A (2015). Perfluorooctanoic acid (PFOA) affects distinct molecular signalling pathways in human primary hepatocytes. Toxicology, 333: 53–62. https://doi.org/10.1016/j.tox.2015.04.004

Conway BN, Badders AN, Costacou T, Arthur JM, Innes KE (2018). Perfluoroalkyl substances and kidney function in chronic kidney disease, anemia, and diabetes. Diabetes, Metab. Syndr. Obes. 11: 707–716. https://doi.org/10.2147/DMSO.S173809

Cui L, Zhou QF, Liao CY, Fu JJ, Jiang GB (2009). Studies on the toxicological effects of PFOA and PFOS on rats using histological observation and chemical analysis. Arch. Environ. Contam. Toxicol., 56: 338–349. https://doi.org/10.1007/s00244-008-9194-6

De Silva AO, Armitage JM, Bruton TA, Dassuncao C, Heiger-Bernays W, Hu XC, Kärrman A, Kelly B, Ng C, Robuck A, Sun M, Webster TF, Sunderland EM (2021). PFAS exposure pathways for humans and wildlife: A synthesis of current knowledge and key gaps in understanding. Environ. Toxicol. Chem., 40: 631–657. https://doi.org/10.1002/etc.4935

Epa (2012). Emerging contaminants Perfluorooctane sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA) At a Glance. pp. 6.

Franko J, Meade BJ, Frasch HF, Barbero M, Anderson SE (2012). Dermal penetration potential of perfluorooctanoic acid (PFOA) in human and mouse skin. J. Toxicol. Environ. Health A Curr. Issues, 75: 50–62. https://doi.org/10.1080/15287394.2011.615108

Havakhah S, Sadeghnia HR, Hajzadeh MAR, Roshan NM, Shafiee S, Hosseinzadeh H, Mohareri N, Rad AK (2014). Effect of *Nigella sativa* on ischemia-reperfusion induced rat kidney damage. Iran. J. Basic Med. Sci., 17: 986–992.

Hidayat M, Prahastuti S, Delima ER, Setiawati L, Soemardji AA (2017). High doses of soybean, jati belanda and their combination extracts have no acute toxic effects. Health Sci. J. Indones., 8. https://doi.org/10.22435/hsji.v8i2.7381.124-132

Hossain MS, Rahman MZ, Mozumder SN, Rahman J (2024). Nutritional composition, fatty acids, bioactive compounds, and antioxidant activity of *Nigella sativa* seed grown in Bangladesh. Discov. Food, 4. https://doi.org/10.1007/s44187-024-00102-x

Li Y, Fletcher T, Mucs D, Scott K, Lindh CH, Tallving P, Jakobsson K (2018). Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. Occup. Environ. Med., 75: 46–51. https://doi.org/10.1136/oemed-2017-104651

Liu D, Yan S, Wang P, Chen Q, Liu Y, Cui J, Liang Y, Ren S, Gao Y (2023). Perfluorooctanoic acid (PFOA) exposure in relation to the kidneys: A review of current available literature. Front. Physiol., 14: 1103141. https://doi.org/10.3389/fphys.2023.1103141

Majdalawieh AF, Fayyad MW, Nasrallah GK (2017). Anti-cancer properties and mechanisms of action of thymoquinone, the major active ingredient of *Nigella sativa*. Crit. Rev. Food Sci. Nutr., 57: 3911–3928. https://doi.org/10.1080/10408398.2016.1277971

Miah MA, Himel MH, Sujan KM, Mustari A, Haque MI (2022). Protective effects of cinnamon powder against hyperlipidemia and hepatotoxicity in butter fed female albino mice. Saudi. J. Biol. Sci., 22: 3069-3074. https://doi.org/10.1016/j.sjbs.2022.01.047

Mollazadeh H, Hosseinzadeh H (2014). The protective effect of *Nigella sativa* against liver injury: A review. Iran. J. Basic Med. Sci., 17: 958.

Naghsh N, Moridpour AH, Kavyani Z, Musazadeh V, Jafarzadeh J, Safaei E, Clark CCT, Faghfouri AH (2023). The effect of *Nigella sativa* (black seed) supplementation on body weight and body composition: A GRADE-assessed systematic review and dose-response meta-analysis of randomized controlled trials. J. Funct. Foods, 105: 105565. https://doi.org/10.1016/j.jff.2023.105565

Pierozan P, Jerneren F, Karlsson O (2018). Perfluorooctanoic acid (PFOA) exposure promotes proliferation, migration and invasion potential in human breast epithelial cells. Arch. Toxicol., 92: 1729–1739. https://doi.org/10.1007/s00204-018-2181-4

Rahim H, Khan S, Ghani A, Amin F, Jan NU, Khan N (2023). Potential effect of *Nigella sativa* tablets on fatty liver disease in rats. BMC J. Med. Sci., 4: 79–84. https://doi.org/10.70905/bmcj.04.02.0162

Rakib MS, Sujan KM, Miah MA (2021). Deleterious effects of mercuric chloride on blood biochemistry, liver and kidney histology in female albino mice. J. Agric. Food Environ., 2: 18–23. https://doi.org/10.47440/JAFE.2021.2204

Razmpoosh E, Safi S, Abdollahi N, Nadjarzadeh A, Nazari M, Fallahzadeh H, Mazaheri M, Salehi-Abargouei A (2020). The effect of Nigella sativa on the measures of liver and kidney parameters: A systematic review and meta-analysis of randomized-controlled trials. Pharmacol. Res., 156: 104767. https://doi.org/10.1016/j.phrs.2020.104767

Sarker S, Haque MI, Sujan KM, Talukder MI, Miah MA (2019). Curcumin attenuates butter fat induced hyperlipidemia in mice. J. Bangladesh Agric. Univ., 17: 220–225. https://doi.

org/10.3329/jbau.v17i2.41972

Story C (2025). PFOA administration in rats with chronic kidney disease leads to kidney and liver hypertrophy and decreases blood pressure.

Sunderland EM, Hu XC, Dassuncao C, Tokranov AK, Wagner CC, Allen JG (2019). A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. J. Expo. Sci. Environ. Epidemiol., 29: 131–147. https://doi.org/10.1038/s41370-018-0094-1

Suvarna KS, Layton C, Bancroft JD (2018). Bancroft's theory and practice of histological techniques. Elsevier health sciences.

Tiji S, Benayad O, Berrabah M, El Mounsi I, Mimouni M (2021). Phytochemical profile and antioxidant activity of *Nigella sativa* L growing in Morocco. Sci. World J., 2021. https://doi.org/10.1155/2021/6623609

Tsuda S (2016). Differential toxicity between perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). J. Toxicol. Sci., 41: SP27–SP36. https://doi.org/10.2131/jts.41.SP27

Wang L, Sun W, Ma X, Griffin N, Liu H (2024). Perfluorooctanoic acid (PFOA) exposure induces renal filtration and reabsorption disorders via down-regulation of aquaporins. Toxicol. Lett., 392: 22–35. https://doi.org/10.1016/j.toxlet.2023.12.003

Wang Z, DeWitt JC, Higgins CP, Cousins IT (2017). A never-ending story of per-and polyfluoroalkyl substances (PFASs)? https://doi.org/10.1021/acs.est.6b04806

Wen Y, Chen J, Li J, Arif W, Kalsotra A, Irudayaraj J (2020). Effect of PFOA on DNA methylation and alternative splicing in mouse liver. Toxicol. Lett., 329: 38–46. https://doi.org/10.1016/j.toxlet.2020.04.012

Wu X, Liang M, Yang Z, Su M, Yang B (2017). Effect of acute exposure to PFOA on mouse liver cells *in vivo* and *in vitro*. Environ. Sci. Pollut. Res., 24: 24201–24206. https://doi.org/10.1007/s11356-017-0072-5

Zahm S, Bonde JP, Chiu WA, Hoppin J, Kanno J, Abdallah M, Blystone CR, Calkins MM, Dong GH, Dorman DC, Fry R, Guo H, Haug LS, Hofmann JN, Iwasaki M, Machala M, Mancini FR, Maria-Engler SS, Moller P, Ng JC, Pallardy M, PM (2024). Carcinogenicity of perfluorooctanoic acid and perfluorooctanesulfonic acid. Lancet Oncol., 25: 16–17. https://doi.org/10.1016/S1470-2045(23)00622-8