

Impact of Silver Nanoparticles Extract of *Aloe vera* Gel (*Aloe barbadensis* Miller) Induced by Perfluorooctanoic Acid on Genotoxicity

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Alyamani et al.: Genotoxic Impact of *Aloe vera*-Mediated Silver Nanoparticles

The green synthesis of nanoparticles is gaining popularity due to its environmentally friendly, non-toxic and cost-effective properties. The distribution used of microproducts into the world are raised the concern of their implications on public health, in particular, Perfluorooctanoic acid which are variety used in a range of industries and consumer products as surfactants. The current study focused on the effects of biologically synthesized silver nanoparticle extract from *Aloe vera* (*Aloe vera*- silver nanoparticles) on genotoxicity induced by Perfluorooctanoic acid. After the treatment period, the rats were euthanized, blood and liver samples were collected for biochemical, histological and genetic analysis. The results indicate a notable increase in oxidative stress markers (glutathione, superoxide dismutase, catalase and lipid peroxidation), associated with a pathological change in the liver cells and deoxyribonucleic acid damage following treatment with Perfluorooctanoic acid. In contrast, treatment with silver nanoparticle extract of *Aloe vera* gel significantly mitigated oxidative stress, enhanced liver function and decreased deoxyribonucleic acid strand breaks.

Key words: Perfluorooctanoic acid, nanoparticles, the green synthesis, eco-friendly, toxicity, antioxidant

Nanotechnology has profoundly impacted numerous facets of human life, sparking significant enthusiasm in life sciences, especially in biomedical devices and biotechnology^[1]. The unique attributes of nanomaterials, attributed to their tiny size and extensive surface area compared to their volume, set them apart from more significant substances^[2]. These materials exhibit distinct physical, chemical and biological properties that enhance their utility across various applications^[3].

This technology focuses on producing tiny nanoparticles, which are essential for the treatment of diseases in both plants and animals, demonstrating remarkable potential for enhancing human health and overall quality of life^[4]. Green or biogenic synthesis refers to environmentally friendly methods of producing materials, particularly Silver Nanoparticles (AgNPs), through biological such as plants, fungi, algae and bacteria^[5]. These AgNPs exhibit various beneficial properties, including antimicrobial, antiangiogenic, antitumor, anti-inflammatory and antioxidant activities^[6]. Consequently, silver

nanoparticles are commonly employed as active components in numerous biomedical applications. These include serving as vaccine adjuvants in anti-diabetic therapies, cancer treatment, biosensing techniques and repairing wounds and bones^[7,8].

On the other hand, Polyfluoroalkyl Substances (PFAS), often referred to as “forever chemicals” have been used since the 1950s due to their resistance to water and heat. Perfluorooctanoic Acid (PFOA), a prevalent PFAS, contaminates various products and contributes to environmental pollution. Human exposure primarily occurs through food and drinking water^[9]. Recent studies have detected PFOA and other PFAS in the Red Sea’s coastal waters and marine life in Saudi Arabia, underscoring the associated environmental and health hazards from seafood consumption^[10].

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Herbal remedies one noteworthy example is *Aloe vera* (*A. vera*) which has long been lauded for its health benefits, mainly attributable to its anti-inflammatory and antioxidant characteristics^[11]. Studies have demonstrated that *A. vera* can aid in mitigating liver damage by decreasing oxidative stress and inflammation, which are critical contributors to liver disorders^[12]. This plant flourishes in hot, dry climates and can adapt to various environments, such as deserts, grasslands and even coastal or alpine regions^[13]. In Saudi Arabia, it is prevalent, particularly in the desert landscape of the Asir area, where it is locally referred to as “Al-Maguar” or “Sabar”^[14]. Of the over 400 recognized species of Aloe, *Aloe barbadensis* Miller (also categorized as *A. vera* Linne and commonly known as *A. vera*) is the most renowned and extensively utilized. *A. vera* comprises at least 75 bioactive constituents contributing to various biological effects, including anti-inflammatory, antioxidant and antitumor properties^[15]. Research has indicated that *A. vera* extracts can aid in restoring liver function and improving liver health following chemical-induced damage^[16]. Thus, the current research focused on the physiological and histological impact on the liver of male rats by estimating the changes in liver enzymes and oxidative stress parameters induced by

PFOA exposure and evaluating the potential effects of green silver nanoparticle extract of *A. vera* gel. In addition, evaluate the genotoxic effects on the Deoxyribonucleic Acid (DNA) of liver cells by analysing the alterations triggered by PFOA and the possible influence of green silver nanoparticles extracted from *A. vera* gel.

MATERIALS AND METHODS

Chemicals:

PFOA: PFOA (95 % purity, 171468-5G) was obtained from Sigma-Aldrich St. Louis, MO, USA).

Extraction and preparation: Green AgNPs from *A. vera* gel extract, *A. vera* (*Aloe barbadense* Miller) was purchased from the local market in the Jeddah city, Saudi Arabia.

Green silver nanoparticles from *A. vera* preparation and fresh *A. vera* gel were prepared according to Khan *et al.*^[17].

Characterization of AgNPs:

Prepared herbal nanoparticles were confirmed by Atomic Force Microscopy AFM^[18], Dynamic light scattering DLS^[19], X-Ray Diffraction (XRD)^[20] and zeta potential fig. 1-fig. 4^[21].

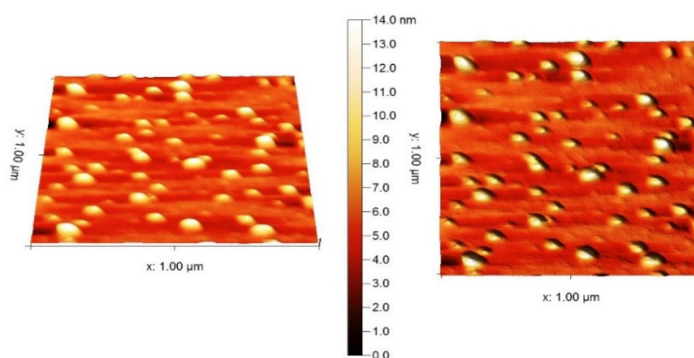


Fig. 1: Atomic Force Microscopy (AFM) of nanoparticles extract of *A. vera* gel

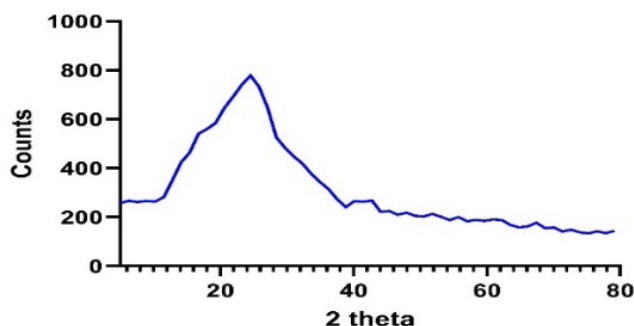


Fig. 2: Dynamic Light Scattering (DLS) of nanoparticles extract of *A. vera* gel

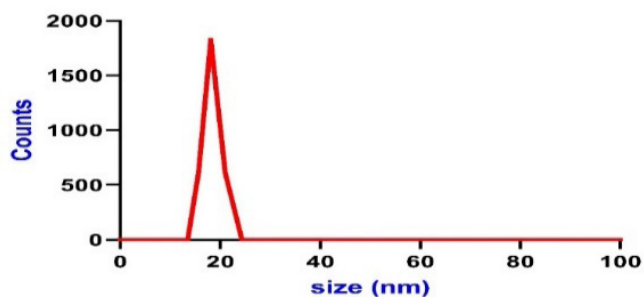


Fig. 3: XRD of nanoparticles extract of *A. vera* gel

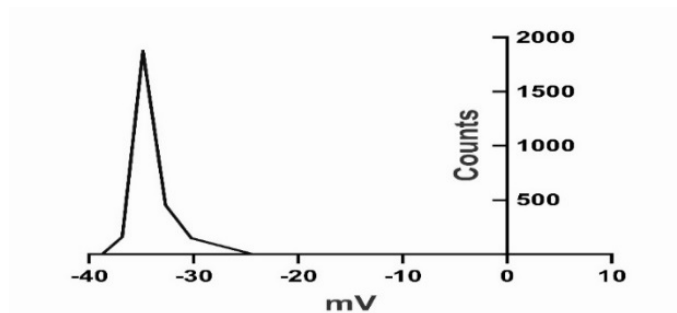


Fig. 4: Zeta Potential (ZETA) of nanoparticles extract of *A. vera* gel

Experimental animals: The animals were 24 adult healthy albino male rats of Wister strain with weights between 140 and 180 g. Animals were obtained from the pharmacy college student's section at King Abdul-Aziz University (KAU), Jeddah, Saudi Arabia.

The experiment period is 6 w. The experiment was completed in the pharmacy college student's section at KAU.

Experimental design: Male albino rats (140±180 g) were used in this study and the animals were acclimated under standard conditions. Rats were randomly classified into six groups (n=6).

G1 (Negative control group): Served as control and received oral administration of distilled water daily for 6 w.

G2 (Positive control group): Rats were treated orally with 25 mg/kg/day PFOA for 6 w^[22].

G3: Rats were treated orally with 150 mg/kg/day nanoparticles extract of *A. vera* gel for 6 w^[23].

G4: Rats were treated with PFOA+nanoparticles extract of *A. vera* gel daily for 6 w.

Biochemical assay:

Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase

(ALP) and bilirubin, liver oxidative stress biomarkers: Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT) and Lipid Peroxidation (LPO) by using the rat Enzyme-Linked Immunosorbent Assay (ELISA) kits obtained from BioSource USA following the teachings and steps included in the research^[24].

Histological assessment:

The liver samples were flooded and fixed in 10 % formalin solution for 48 h, then embedded in paraffin wax; preparing sections stained with Ehrlich's Hematoxylin and Eosin (H&E) was used for histological study, according to Bancroft and Stevens *et al.*^[25].

Genetic procedures: The comet assay kits (3-well slides) obtained from (ab238544).

Statistical analysis:

The mean value±standard error was used to express the data by a t-test for the statistical study to decide if the experiment groups had significant differences. A p-value of <0.05 was considered significant. The Statistical Package for Social Sciences (SPSS) software (version 26, SPSS INC., USA) was used for each statistical analysis.

RESULTS AND DISCUSSION

Table 1 and fig. 5-fig. 8 the current study shows the PFOA group (G2) has levels of liver function enzymes, including ALT (43.60±4.05 U/L), AST (47.91±3.02 U/L), ALP (86.25±3.67 U/L), and bilirubin (1.96±0.15 mg/dl) in the PFOA group (G2) compared to the control group (G1) at $p < 0.05$. In contrast, the group receiving a nanoparticle extract of *A. vera* gel (G3) displays a significant rise in ALP levels, alongside a noteworthy decrease in bilirubin levels relative to the control group (G1) at $p < 0.05$.

Furthermore, treatment with the nanoparticle extract of *A. vera* gel (G4) alongside PFOA results in a significant reduction in liver enzymes: ALT decreases by (15.25±0.99 U/L), AST decreases by (19.41±1.54 U/L), ALP decreases by (47.83±1.92 U/L), and bilirubin decreases by (0.08±0.03 mg/dl) compared to the positive control group (G2) at $p < 0.05$. Suggest that the nanoparticle extract of *A. vera* gel effectively ameliorates liver enzyme levels impacted by PFOA toxicity.

Table 2 and fig. 9-fig. 12 the liver oxidative stress analysis results across different study groups reveal the following findings: In the PFOA treatment group (G2), there is a significant decrease in levels of GSH, measured at (4.18±0.39 ng/ml), as well as SOD at (95.75±3.56 u/ml) and CAT at (84.50±2.48 U/ml). Also, there is a marked increase in LPO, recorded at (7.45±0.63 nmol/ml) compared to the control group (G1), with statistical significance noted ($p < 0.05$).

In the group treated with the nanoparticle extract of *A. vera* gel (G3), there is a significant decrease in oxidative stress as indicated by SOD levels at (156.17±1.34 μ /ml) and a notable improvement in

LPO, measured at (2.88±0.46 nmol/ml), compared to the control group (G1) ($p < 0.05$).

Meanwhile, in the group receiving both PFOA and the nanoparticle extract of *A. vera* gel (G4), there is a significant decrease in LPO at (2.87±0.31 nmol/ml), along with substantial increases in CAT levels at (129.16±2.94 U/ml), GSH at (14.36±0.92 ng/ml) and SOD at (175±3.65 u/ml) compared to the PFOA positive control group (G2) ($p < 0.05$). It suggests that the nanoparticle extract of *A. vera* gel effectively ameliorates. Specifically, the extract enhances the activity of key antioxidant enzymes impacted by PFOA toxicity.

Histological analysis of liver sections from the control group (G1) present a typical structure (fig. 13A). In contrast, examination of liver tissue treated with PFOA at 25 mg/kg/day shows notable alterations in the portal tract, including the infiltration of inflammatory cells in the surrounding area. Furthermore, changes in the Bile Ducts (BD) present as inflammation, cellular necrosis, increased arterial wall thickness, and blood clots. Additionally, the Central Veins (CV) appear constricted and compact in shape (fig. 13B).

The liver section treated with a nanoparticle extract of *A. vera* gel (G3) (150 mg/kg/day) shows no changes, as illustrated in fig. 13C. Upon examination of the liver section, significant alterations are noted due to treatment with PFOA and the nanoparticle extract of the *A. vera* gel group (G4). Mild inflammation is detected, particularly in the portal tract, containing inflammatory cells. The CV exhibits a few inflammatory cells and mild fibrosis, as depicted in fig. 13D.

TABLE 1: IMPACT OF 6 W DAILY ADMINISTRATION OF PFOA (25 mg/kg/day), NANOPARTICLES EXTRACT OF *A. vera* GEL (150 mg/kg/day), AND PFOA IN CONJUNCTION WITH NANOPARTICLE EXTRACT OF *A. vera* GEL ON ALT (U/L), AST (U/L), ALP (U/L) AND BILIRUBIN (mg/dl) LEVELS IN ADULT MALE ALBINO RATS COMPARED TO CONTROL

Groups\Variables	ALT (U/L)	AST (U/L)	ALP (U/L)	Bilirubin (mg/dl)
G1	12.59±0.51	16.16±0.69	42.33±0.84	0.73±0.08
G2	43.60±4.05*	47.91±3.02*	86.25±3.67*	1.96±0.15*
G3	19.25±1.43	23.79±1.86	57.08±5.24*	0.62±0.05*
G4	15.25±0.99#	19.41±1.54#	47.83±1.92#	0.08±0.03#

Note: Data was expressed as mean±standard error of the mean, significance groups made by t-test. * $p < 0.05$: Significance compared to control and # $p < 0.05$: Significance compared to positive control

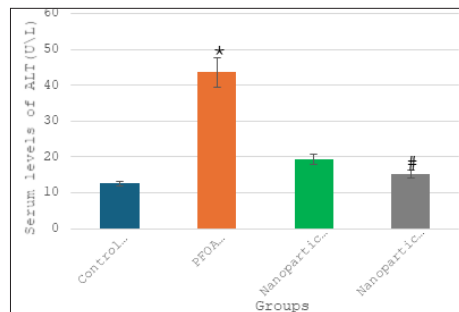


Fig. 5: Impact of 6 w Daily Administration of PFOA (25 mg/kg/day), nanoparticles extract of *A. vera* gel (150 mg/kg/day), and PFOA in conjunction with Nanoparticle Extract of *A. vera* gel on ALT(U/L) level in adult male albino rats compared to control
Note: Data was expressed as mean±standard error of the mean, significance groups made by t-test. *p<0.05: Significance compared to control and #p<0.05: Significance compared to positive control, (■): Control group (G1); (■): PFOA (G2); (■): Nanoparticle Extract of *A. vera* gel (G3) and (■): Nanoparticle Extract of *A. vera* gel (G4)

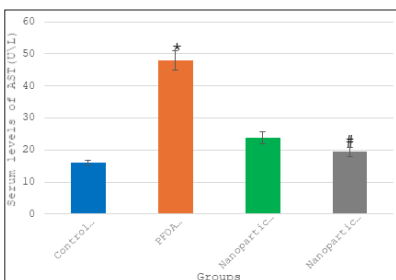


Fig. 6: Impact of 6 w daily administration of PFOA (25 mg/kg/day), nanoparticles extract of *A. vera* gel (150 mg/kg/day), and PFOA in conjunction with nanoparticle extract of *A. vera* gel on AST (u/l) level in adult male albino rats compared to control
Note: Data was expressed as mean±standard error of the mean, significance groups made by t-test. *p<0.05: Significance compared to control and #p<0.05: Significance compared to positive control, (■): Control group (G1); (■): PFOA (G2); (■): Nanoparticle Extract of *A. vera* gel (G3) and (■): Nanoparticle Extract of *A. vera* gel (G4)

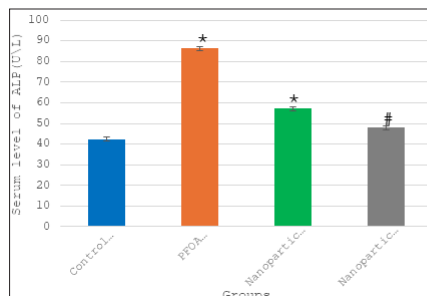


Fig. 7: Impact of 6 w Daily Administration of PFOA (25 mg/kg/day), nanoparticles extract of *A. vera* Gel (150 mg/kg/day), and PFOA in conjunction with nanoparticle extract of *A. vera* gel on ALP (u/l) level in adult male albino rats compared to control
Note: Data was expressed as mean±standard error of the mean, significance groups made by t-test. *p<0.05: Significance compared to control and #p<0.05: Significance compared to positive control, (■): Control group (G1); (■): PFOA (G2); (■): Nanoparticle Extract of *A. vera* gel (G3) and (■): Nanoparticle Extract of *A. vera* gel (G4)

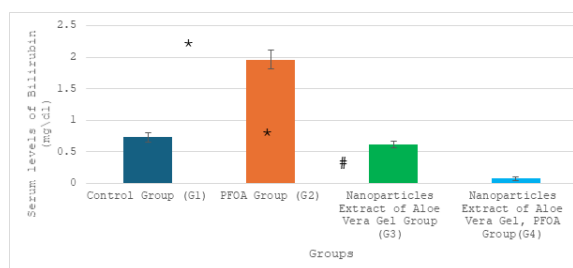


Fig. 8: Impact of 6 w Daily Administration of PFOA (25 mg/kg/day), nanoparticles extract of *A. vera* gel (150 mg/kg/day), and PFOA in conjunction with nanoparticle extract of *A. vera* gel on bilirubin (mg/dl) level in adult male albino rats compared to control
Note: Data was expressed as mean±standard error of the mean, significance groups made by t-test. *p<0.05: Significance compared to control and #p<0.05: Significance compared to positive control, (■): Control group (G1); (■): PFOA (G2); (■): Nanoparticle Extract of *A. vera* gel (G3) and (■): Nanoparticle Extract of *A. vera* gel (G4)

TABLE 2: EFFECT OF DAILY ADMINISTRATION OF PFOA (ORAL WITH 25 mg/kg/day), NANOPARTICLES EXTRACT OF *A. vera* GEL (ORAL WITH 150 mg/kg/day), AND PFOA IN CONJUNCTION WITH NANOPARTICLE EXTRACT OF *A. vera* GEL FOR 6 w COMPARED TO CONTROL IN GSH (ng/ml), SOD (μ ml), CAT (μ l), AND LPO (nmol/ml) LEVELS IN ADULT MALE ALBINO RATS

Groups\Variables	GSH (ng/ml)	SOD (μ ml)	CAT (μ l)	LPO (nmol/ml)
G1	17.10 \pm 0.9	173.50 \pm 3.90	121.10 \pm 1.1	1.65 \pm 0.1
G2	4.18 \pm 0.39*	95.75 \pm 3.56*	84.50 \pm 2.48*	7.45 \pm 0.63*
G3	17.08 \pm 1.34	156.17 \pm 1.34*	116.76 \pm 1.42	2.88 \pm 0.46*
G4	14.36 \pm 0.92#	175 \pm 3.65#	129.16 \pm 2.94#	2.87 \pm 0.31#

Note: Data was expressed as mean \pm standard error of the mean, significance groups made by t-test. *p<0.05: Significance compared to control and #p<0.05: Significance compared to positive control

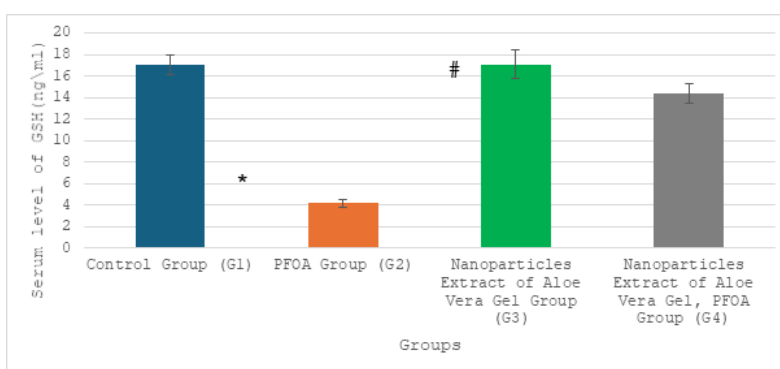


Fig. 9: Effect of daily administration of PFOA (Oral with 25 mg/kg/day), nanoparticles extract of *A. vera* gel (Oral with 150 mg/kg/day), and PFOA in conjunction with nanoparticle extract of *A. vera* gel for 6 w compared to control in SOD (μ ml) level in adult male albino rats

Note: Data was expressed as mean \pm standard error of the mean, significance groups made by t-test. *p<0.05: Significance compared to control and #p<0.05: Significance compared to positive control, (■): Control group (G1); (■): PFOA (G2); (■): Nanoparticle Extract of *A. vera* gel (G3) and (■): Nanoparticle Extract of *A. vera* gel (G4)

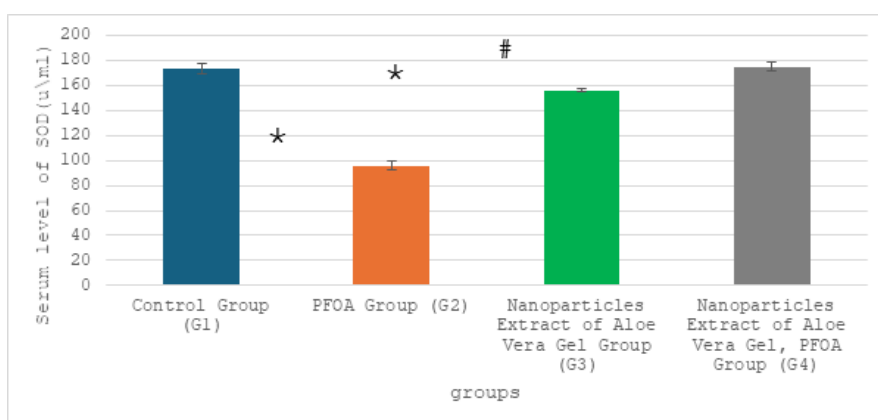


Fig. 10: Effect of Daily Administration of PFOA (Oral with 25 mg/kg/day), nanoparticles extract of *A. vera* gel (Oral with 150 mg/kg/day), and PFOA in conjunction with nanoparticle extract of *A. vera* gel for 6 w compared to control in CAT (μ l) Level in adult male albino rats

Note: Data was expressed as mean \pm standard error of the mean, significance groups made by t-test. *p<0.05: Significance compared to control and #p<0.05: Significance compared to positive control, (■): Control group (G1); (■): PFOA (G2); (■): Nanoparticle Extract of *A. vera* gel (G3) and (■): Nanoparticle Extract of *A. vera* gel (G4)

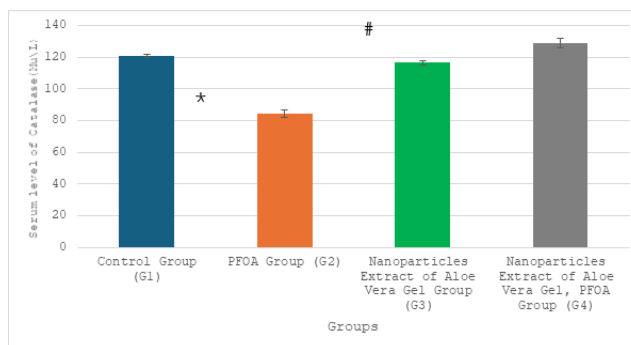


Fig. 11: Effect of Daily Administration of PFOA (Oral with 25 mg/kg/day), nanoparticles extract of *A. vera* gel (Oral with 150 mg/kg/day), and PFOA in conjunction with nanoparticle extract of *A. vera* gel for 6 w compared to control in CAT (muL) Level in adult male albino rats

Note: Data was expressed as mean±standard error of the mean, significance groups made by t-test. *p<0.05: Significance compared to control and #p<0.05: Significance compared to positive control, (■): Control group (G1); (■): PFOA (G2); (■): Nanoparticle Extract of *A. vera* gel (G3) and (■): Nanoparticle Extract of *A. vera* gel (G4)

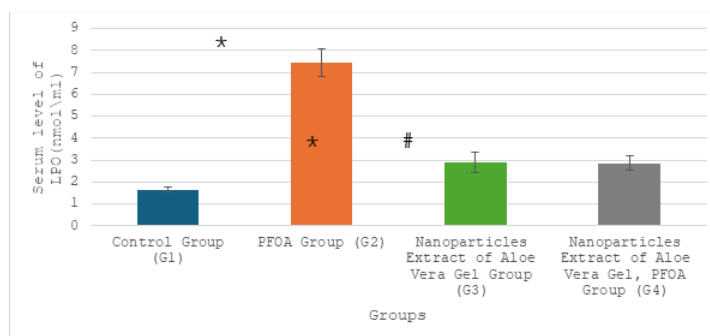


Fig. 12: Effect of daily administration of PFOA (Oral with 25 mg/kg/day), nanoparticles extract of *A. vera* gel (Oral with 150 mg/kg/day), and PFOA in conjunction with nanoparticle extract of *A. vera* gel for 6 w compared to control in LPO (nmol/ml) level in adult male albino rats

Note: Data was expressed as mean±standard error of the mean, significance groups made by t-test. *p<0.05: Significance compared to control and #p<0.05: Significance compared to positive control, (■): Control group (G1); (■): PFOA (G2); (■): Nanoparticle Extract of *A. vera* gel (G3) and (■): Nanoparticle Extract of *A. vera* gel (G4)

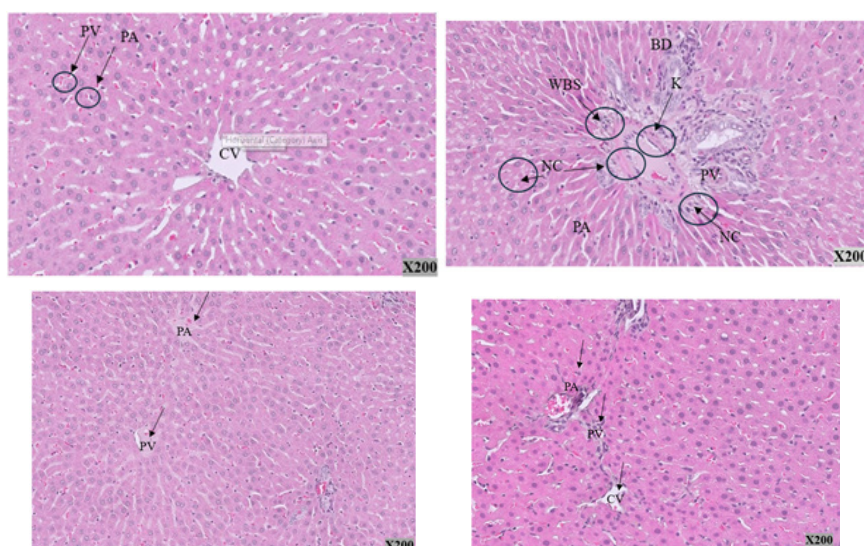


Fig. 13: (A): Light microphotograph of liver section from rat in control Group (G1) showing normal Portal Vein (PV) and Artery (PA), and Central Vein (H&E Staining; Magnification X200); (B): Light microphotograph of the liver section from a rat treated with PFOA (G2) for 6 w shows the Portal Vein (PV), Artery (PA), Bile Duct (BD) Central Vein (CV), Necrotic Cell (NC), Kupffer Cell (K), and Blood Sinusoids (WBS) (Black Circles) (H&E Staining; Magnification X200); (C): Light microphotograph of liver section from a rat treated with nanoparticles extract of *A. vera* gel (G3) for 6 w show the Portal Vein (PV), Artery (PA) (Black Arrows) (H&E Staining; Magnification X200) and (D): Light microphotograph of a liver section from rats treated with PFOA and nanoparticles extract of *A. vera* gel (G4) for 6 w Central Vein (CV) and Portal Vein (PV), and Portal Artery (PA) (Black Circle) (H&E staining; magnification X200)

Table 3 and fig. 14 illustrate the effects of PFOA, nanoparticle extracts of *A. vera* gel, and PFOA combined with nanoparticle extracts from *A. vera* gel on DNA head and tail lengths. The results show that the PFOA group (G2) demonstrates a significant reduction in head length (99.46 ± 0.29) and a substantial increase in tail length (354.40 ± 1.7) compared to the control group (G1), with statistical significance at $p < 0.05$.

The group treated with nanoparticle extracts of *A. vera* gel (G3) exhibits a notable decrease in head length (218.66 ± 0.20) and a minor increase in tail length (14.80 ± 0.32) compared to the control group, also at $p < 0.05$.

Furthermore, the group receiving both PFOA and nanoparticle extracts from *A. vera* gel (G4) shows a significant increase in head length (225.53 ± 0.3) along with a decrease in tail length (17.06 ± 0.12) compared to the PFOA-Positive group (G2), with statistical significance at $p < 0.05$. The nanoparticle extracts of *A. vera* gel suggest a considerable reduction in DNA

strand breaks or damage.

PFOA is a persistent environmental pollutant widely used in various industrial applications. Evidence has shown that PFOA exposure disrupts enzymatic functions, promotes oxidative stress, and induces tissue and genetic damage, ultimately contributing to chronic health risks, particularly hepatic dysfunction^[26]. After ingestion, PFOA is transported through the bloodstream to the liver, which undergoes metabolic processing and excreted *via* bile^[27,28]. This hepatic burden frequently results in hepatocellular injury and impaired liver function.

Serum levels of liver enzymes such as ALT, AST, ALP, and bilirubin serve as essential biomarkers for liver damage. Elevated levels indicate hepatocyte membrane disruption or bile duct obstruction^[29,30]. In agreement with previous studies^[31,32], our findings demonstrate significant liver enzyme (ALT, AST, ALP, and Bilirubin) elevations following oral administration of PFOA (25 mg/kg/day for 6 w), confirming its hepatotoxic effects.

TABLE 3: EFFECTS OF DAILY ADMINISTRATION OF PFOA (25 mg/kg/day), NANOPARTICLE EXTRACT OF *A. vera* GEL (150 mg/kg/day), AND PFOA IN CONJUNCTION WITH NANOPARTICLE EXTRACT OF *A. vera* GEL FOR 6 W ON DNA HEAD AND TAIL LENGTHS IN ADULT MALE ALBINO RATS COMPARED TO CONTROL

Group	DNA head length Mean \pm SE	DNA tail length Mean \pm SE
G1	230.15 \pm 0.82	11.76 \pm 0.14
G2	99.46 \pm 0.29*	354.40 \pm 1.7*
G3	218.66 \pm 0.20*	14.80 \pm 0.32*
G4	225.53 \pm 0.3#	17.06 \pm 0.12#

Note: Data was expressed as mean \pm standard error of the mean, significance groups made by t-test. * $p < 0.05$: Significance compared to control and # $p < 0.05$: Significance compared to positive control

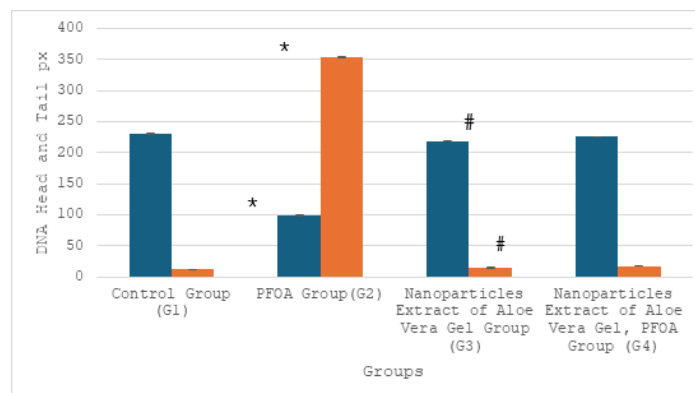


Fig. 14: Effects of daily administration of PFOA (25 mg/kg/day), nanoparticle extract of *A. vera* gel (150 mg/kg/day), and PFOA in conjunction with nanoparticle extract of *A. vera* Gel for 6 w on DNA head and tail lengths in adult male albino rats compared to control

Note: Data was expressed as mean \pm standard error of the mean, significance groups made by t-test. * $p < 0.05$: Significance compared to control and # $p < 0.05$: Significance compared to positive control, (■): DNA head length and (■): DNA tail lengths

Study of Yang *et al.*^[33] highlights the links between PFOA exposure and hepatic toxicity in animal models, underscoring how oxidative stress can lead to LPO and subsequent hepatocyte injury this body of work emphasizes the need for continued monitoring and research into the long-term health effects of PFOA exposure and the mechanisms by which it exerts its toxic effects on the liver Oxidative stress mechanisms demonstrate that elevated levels of stress-related Endoplasmic Reticulum (ER) proteins in response to PFOA exposure lead to ER stress. Additionally, PFOA impairs mitochondrial function within liver tissue, further exacerbating liver damage. This is particularly concerning due to the crucial relationship between the ER and mitochondria in regulating cellular metabolism, signalling pathways, and maintaining overall homeostasis^[34].

Oxidative stress is central to PFOA-induced liver damage. PFOA increases Reactive Oxygen Species (ROS) production in hepatocytes, leading to LPO and DNA damage^[35]. PFOA exposure impairs Mitochondria, primary ROS sources, which exacerbate hepatotoxic effects^[36,37]. Antioxidants like GSH, SOD, and CAT protect cells by neutralizing ROS^[38]. However, PFOA depletes these antioxidants, impairing mitochondrial function and increasing oxidative stress markers such as LPO^[39,40].

Our results corroborate these findings, showing significant reductions in GSH, SOD, and CAT levels alongside increased LPO after PFOA treatment ($p < 0.05$).

Moreover, high doses of PFOA increase LPO and decrease vital antioxidant enzymes like SOD and GSH Peroxidase (GSH-PX), which are crucial for free radical scavenging^[41]. In our study, liver samples from PFOA-treated rats showed significant reductions in SOD and GSH-PX compared to controls. Maintaining the oxidant-antioxidant balance is essential for regulating key cellular processes, including proliferation and apoptosis^[42,43]. Overall, our findings align well with previous research, supporting PFOA's established hepatotoxic and oxidative stress-related effects.

Our study shows that oral PFOA treatment (25 mg/kg/day for 6 w) causes notable liver histopathological changes, such as portal tract inflammation, bile duct necrosis, arterial wall thickening with clots, and central vein narrowing. These alterations are in line with the findings of^[33], suggesting that inflammatory responses, possibly driven by hepatocyte-released

cytokines, play a significant role in liver injury and may sensitize the tissue to other toxins.

Recent studies further associate PFOA with hepatocyte apoptosis, altered bile acid metabolism, and increased adipogenesis^[44,45]. PFOA also increases ROS levels, promoting oxidative stress and apoptosis^[46].

Our results demonstrate that oral administration of PFOA at 25 mg/kg/day resulted in a notable decrease in head length and a significant increase in tail length of the DNA compared to the control group at $p < 0.05$. This aligns with findings in HepG2 cells, where PFOA increased DNA strand breaks in a dose-dependent manner^[47,48]. Further, PFOA-induced oxidative stress, driven by elevated ROS, contributes to DNA damage, mitochondrial dysfunction, and LPO^[49,50]. Increased micronuclei frequency and reduced antioxidant capacity confirm its genotoxicity^[49]. Animal studies also report similar DNA damage in liver tissue at high PFOA doses^[51,52].

These results confirm that PFOA induces hepatotoxicity primarily through oxidative stress, elevated liver enzymes, decreased antioxidant defenses, and DNA damage. These molecular disruptions impair liver function and cause tissue alterations, driven by elevated LPO and decreased antioxidant capacity in rat liver. Ongoing research highlights the serious risks PFOA poses to liver health.

Research highlights the effectiveness of plant extracts in eliminating ROS, with nanoparticles exhibiting enhanced antioxidant capacity due to their larger surface area, which helps minimize DNA damage^[53,54].

In parallel, increasing interest has been directed toward plant-derived antioxidants, including nanoparticle formulations, for mitigating oxidative stress. *A. vera* gel, rich in phytochemicals such as flavonoids, anthraquinones, and vitamins, has demonstrated potent antioxidant, anti-inflammatory, and hepatoprotective effects^[55,56]. Nanoparticle extracts of *A. vera* gel enhance these effects due to their higher surface area and improved bioavailability^[57,58].

Oral administration of nanoparticles extract of *A. vera* gel at 150 mg/kg/day significantly enhances liver enzyme levels in PFOA-treated groups compared to the control group ($p < 0.05$).

This aligns with previous research reporting the protective effects of *A. vera* against chemically induced liver damage, including CCl₄ toxicity^[23].

Silver nanoparticles synthesized using *A. vera* have also demonstrated hepatoprotective effects, potentially through antioxidant-rich compounds such as anthraquinones and flavonoids^[59,60]. These nanoparticles promote parenchymal cell healing and neutralize ROS, which may account for the observed enzyme normalization in our study.

Current research demonstrates a reduction in GSH activity, alongside increased activities of CAT and SOD, and decreased LPO in liver tissues after oral treatment with *A. vera* gel nanoparticles at a dosage of 150 mg/kg/day. This suggests that *A. vera* has a significant impact on alleviating oxidative stress in the liver.

Research suggests that nanoparticles extract of *A. vera* gel may enhance the activities of key antioxidant enzymes, including SOD and GPX. Notably, in cases of chemical-induced toxicity, there is a marked decrease in the activities of SOD, CAT, and GSH in liver tissues, but treatment with silver nanoparticles (AV-AgNPs) has been shown to reverse this decline^[61,62].

Recent findings by Nauroze *et al.*^[23] highlight the added advantages of employing silver nanoparticles synthesized with *A. vera*, as the plant not only aids in reducing silver ions to form nanoparticles but also inhibits the release of these ions through its phytochemical properties, providing improved defense against oxidative stress and toxicity. Seif *et al.*^[63] reports that the normalization of liver enzyme levels after AV-AgNP treatment suggests the recovery and regeneration of hepatocytes within the liver tissue.

Biochemical and histological analyses show that AV-AgNPs effectively protect against oxidative stress by reducing LPO and restoring liver function. Their antioxidant and free radical scavenging properties help normalize liver parameters, making AV-AgNPs promising for treating hepatotoxicity. Plant-derived silver nanoparticles also exhibit enhanced synergistic antioxidant effects^[64].

Compared to the control group, our study demonstrates the effects of orally administered nanoparticle extracts of *A. vera* gel (at a dose of 150 mg/kg/day) on liver damage caused by PFOA exposure. The findings revealed that these

nanoparticles significantly reduced liver damage, as evidenced by mild inflammation and limited tissue damage. Histopathological analysis showed that hepatic tissue exhibited minimal fibrosis and only a few inflammatory cells surrounding the portal tract.

Previous studies support the hepatoprotective effects of *A. vera*, especially in nanoparticle form, through antioxidant and anti-inflammatory actions that reduce LPO and oxidative damage^[65,12]. *A. vera* nanoparticles also limit hepatocyte proliferation and fibrosis, improving liver histology under toxic conditions^[66]. As evidenced by our findings, *A. vera* improved liver structure and reduced lipid accumulation, as Abubakar *et al.*^[67] observed.

The current study illustrates the effect of administering nanoparticle extract of *A. vera* gel orally (150 mg/kg/day). The results revealed a significant increase in head length and a reduction in tail length of DNA compared to the control group ($p < 0.050$), indicating reduced DNA fragmentation. Studies confirm *A. vera* Geno protective effects through its antioxidant components, which reduce DNA damage and maintain genomic stability under oxidative stress^[68,69]. Its nanoparticles neutralize ROS due to antioxidants like vitamins A, C, GSH peroxidase, and SOD, helping preserve DNA integrity. *A. vera* reduced AFB1-induced DNA fragmentation in mice, like the genotoxicity caused by PFOA. These findings are consistent with our results, which showed that *A. vera* nanoparticles significantly reduced DNA damage in PFOA-treated groups, supporting its role as a protective agent against oxidative DNA injury.

In conclusion, oral administration of nanoparticle extract of *A. vera* gel significantly mitigated PFOA-induced hepatotoxicity by reducing liver enzyme levels, restoring antioxidant defenses, improving histological architecture, and protecting DNA integrity. These findings underscore the therapeutic potential of *A. vera* nanoparticles as a natural, plant-based strategy for preventing oxidative liver injury.

Future research should further explore the long-term safety, dose optimization, and pharmacodynamics of *A. vera* nanoparticles in various toxicological models. Investigating their use with other antioxidants or standard hepatoprotective drugs may offer synergistic benefits. Moreover, preclinical trials and molecular studies are warranted to validate these findings and support the clinical translation of *A.*

vera nanoparticle-based therapies.

Ethical approval:

The experimental animals were used rigidly with the rules and principles established by the Research Ethics Committee at King Abdul-Aziz University (KAU). Ethics committee protocol approval No: p115-2023.

Conflict of interests:

The authors declared no conflict of interests.

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