Toxico-pathological effects of perfluorooctanoic acid (PFOA) in normal and diabetic male guinea pigs following oral exposure

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Abstract

Researchers have reported the global distribution and the hazards of perfluorooctanoic acid (PFOA) in human and wildlife. Recently, the relationships between PFOA exposure and surge in total cholesterol, triglyceride, LDL-C, HDL-C and diabetes were detected in workers. However, rodents are probably a poor model for studying PFOA toxicity regarding lipid disturbance following human exposure due to the reverse effects.

The goal of the present study is to present a useful animal toxicity model to evaluate the disturbance of lipid and glucose concentrations associated with increased blood levels of PFOA for 4 weeks.

Total of 40 male guinea pigs were randomly selected and grouped into four equal groups. The first group (G1) served as the control. The second group (G2) was alloxan induced diabetic. The third group (G3) was exposed to PFOA at 100 mg/kg BW orally. Group four (G4) was diabetic guinea pig exposed to PFOA at 100 mg/kg BW.

Serum blood glucose, lipid profiles and histopathological changes in the pancreas, liver and kidney were evaluated.

The results showed that fasting blood glucose and lipid profiles concentrations were significantly increased ($p<0.05$) in G3 and G4 compared with control (G1) and diabetic group (G2). Animals treated with PFOA showed histopathological alterations such hepatic steatosis with signet ring appearance, necrotic alteration of renal tubular epithelium and coalescing Langerhans islet with prominent pancreatic cell hyperplasia.

In conclusion, after 4 weeks, positive associations of PFOA with serum glucose, have been observed in non- diabetic and diabetic animals exposed to PFOA. Further, administration of PFOA caused histopathological changes in the pancreas, liver and kidney in the diabetic and non-diabetic animals. Our results supported the assumption that disturbance in serum lipid and glucose concentrations following PFOA exposure in guinea pigs can mimic a realistic human exposure situation.

Keywords: *Guinea pigs, Perfluorooctanoic acid, Lipid profile, Environmental contamination.*

INTRODUCTION

The perfluoroalkylated substances (PFAS) are large group of chemical compounds that consist of a completely fluorinated hydrophobic alkyl chain with different carbon atoms (from 4 - 16 carbon atoms) and hydrophilic end. Perfluoroalkyl carboxylic acids (PFCAs) are the main subclasses of PFAS. The most prominent member among the perfluoroalkyl carboxylic acids (PFCAs), is perfluorooctanoic acid (PFOA) with eight carbon chain (Larsen and Giovalle, 2015, Liu et al., 2021). Food,

drinking water, industrial manufacturing and commercial products as carpeting, clothing, Gore-Tex, Teflon and paper products are considers the common exposure sources of PFOA (Steenland et al., 2010, Teaf et al., 2019). Perfluorooctanoic acid (PFOA) used for production of fluoropolymer, as surface treatment agents and for the synthesis of other fluorinated polymers. However, there is an evidence that PFOA and its salts possess a number of dangerous characteristics that would be harmful to both the environment and living organisms (Gabbert, 2018). In spite of the fact that, the manufacturing of PFOA has been diminished in recent years, this chemical represent a health and hazard concern due to its persistent in household and commercial goods that produced prior to initiation the program of stewardship (Everds and Kennedy, 2015). Perfluorooctanoic acid (PFOA) has been implicated in various toxicities including neurotoxicity, genotoxicity, nephrotoxicity, epigenetic toxicity, immunotoxicity, reproductive toxicity, and hepatotoxicity (Rashid et al., 2020). Strong evidence indicated the relationship between PFOA exposure and diabetes in workers at fluorinated production sites (Chung et al., 2022). Furthermore, PFOA was related with surge in total cholesterol, high-density lipoprotein-cholesterol (HDL-C) and fasting glucose level (Liu et al., 2018, Louisse et al., 2020, Dunder et al., 2022).

Although previous studies showed that exposure to PFOA can lead to disturbance in metabolism of glucose and increased sensitivity of insulin in mice and rats (Yan et al., 2015), these animal models exhibited opposite results that reported in human regarding lipid disturbance. This study aimed to use male guinea pigs as an experimental animal model to determine the toxico-pathological effect of PFOA in normal and diabetic animals after 4 weeks of exposure.

Materials and Methods

Chemicals

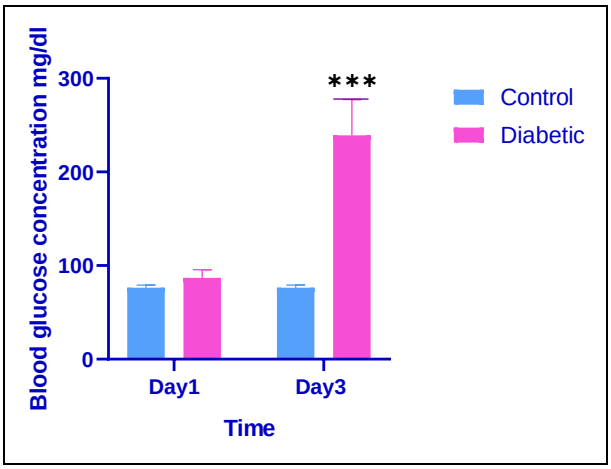
Perfluorooctanoic acid (PFOA), 95% purity, CAS No. 335–67-1 and alloxan monohydrate 98%, CAS No. 2244-11-3 were purchased from Sigma-Aldrich (Saint Louis, USA).

Induction of diabetes

Freshly prepared solution of alloxan monohydrate 5% was used. The solution was prepared by taking (2000 mg) of alloxan monohydrate powder and dissolving in (20 ml) of 0.9% normal saline to get a concentration of (100 mg/ml). Following overnight fasting, guinea pigs were injected intraperitoneally with 200 mg/kg of alloxan monohydrate in 24 hr intervals in order to induce diabetic and maintain the elevated blood sugar levels in the experimental diabetic guinea pigs. Blood glucose test was performed before and after the injection as described in (Akunneh and Aduema, 2018, Al-Hamdani, 2019, Athraa, 2022) with modification.

Diabetes mellitus was induced in twenty guinea pigs represented in G2 and G4, respectively. Guinea pigs with fasting blood glucose levels over 200 mg/dl were considered as diabetic and involved in the experimental study (Figure 1) (Aslan et al., 2013).

Figure 1: Fasting blood glucose concentrations to induce diabetes in guinea pigs, Mean ± SE, *significant differences (*p< 0.05), n=20.



Preparation of PFOA solution

Perfluorooctanoic acid solution was prepared by dissolving 1000 mg in sterile normal saline then, completed to 20 ml sterile normal saline to get concentration of 50 mg/ml. Two groups G3 and G4 of guinea pigs were administered fresh prepared solution at a dose of 100 mg/kg body weight orally/by stomach gavage to induce toxic effect.

Experimental design

Experimental animals (40 guinea pigs/male) were divided randomly into 4 groups, 10 guinea pigs in each group. Group 1: represented as a negative control which receiving normal water ad libitum. Group 2: induced-diabetic male guinea pigs which served as positive control. Animals in group 3: were received 100 mg/kg BW of PFOA orally using stomach gavage. Group 4: induced- diabetic male guinea pigs were received 100 mg/kg BW dose of PFOA orally using stomach gavage. All treatments were conducted 7 days/per week for 4 weeks. This study approved for care and use of laboratory animals from the ethics committee in the pathology department/ veterinary medicine collage/ Baghdad University (Aboktifa & Abbas, 2020).

Blood collection procedure

The first blood collecting from the heart was performed immediately before the 1st dosing and then animals in each group had blood collecting every week after exposure at (1,2,3 and 4 weeks). Blood samples (2.5ml) were collected from each animal by the heart (cardiac puncture) (Surour et al., 2022). Blood was deposited into tube without anticoagulant and then the blood samples were centrifuged at (3000 rpm) for 15 minutes, the serum samples were stored in polyethylene Eppendorf tubes at (-20ºC) (Al-Rikabi, 2012).

Lipid and glucose analysis

The serum concentrations of glucose, total cholesterol, triglyceride, high density lipoprotein-cholesterol HDL-C and low-density lipoprotein cholesterol LDL-C were measured by using spectrophotometric methods. Laboratory kits (SPINREACT, Spain) were used for glucose and lipid profile analysis and their absorbance were read by using spectrophotometer. The intensity of the color formed is proportional to the glucose and lipid concentrations in the sample.

Histopathological evaluation

The guinea pigs were sacrificed with an overdose of a solution containing ketamine (95 mg/ml) and xylazine (5 mg/ml) (Liu et al., 2016). Tissues were collected from the liver, kidney and pancreas. Samples were appropriately prepared for histopathological assessment. 10% neutral buffered formalin was used for samples fixation (Al-Dujaily, 2012) , at this point sample processing performed by automatic tissue processor followed by production paraffin wax blocks of 5 micron by rotary microtome, then stained by hematoxylin

and eosin (Luna, 1968). All the micro-pathological changes in treatment groups were assessed and compared with the control group.

Statistical analysis

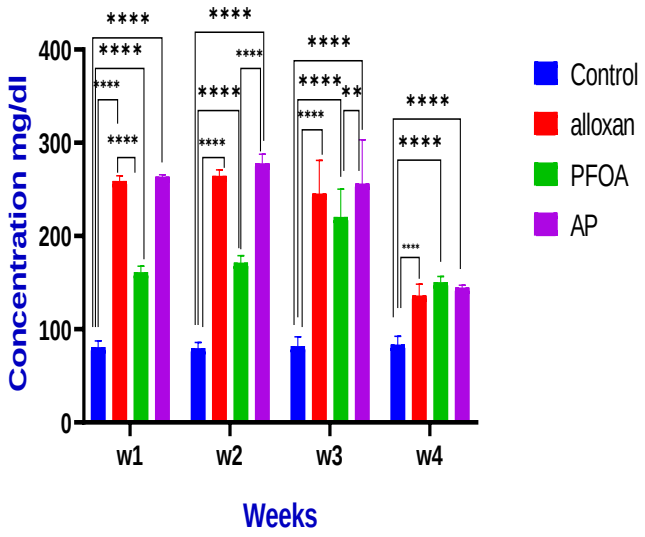
The statistical analysis of the data was carried by using the Graph pad prism Statistical (version 8.0.2). Two-way ANOVA and Tukey's multiple comparisons test were performed to assess significant differences among means of the groups. The results were expressed as mean ± stander errors and P < 0.05 was considered statistically significant.

Results and discussion

Fasting blood sugar test

The results showed that the blood glucose concentrations were significantly increased (p <0.05) in G3 that dosed with PFOA and G4 (diabetic animals that dosed with PFOA) compared with G1 and G2 respectively, during the experimental period of 4 weeks as displayed in Figure 2. These results are in accordance with Yan et al. (2015) who reported that PFOA exposure induced insulin sensitivity and glucose tolerance in mice following 28 day. In another study an elevation in the blood glucose levels after oral exposures to of PFOA were seen in adult male Balb/c mice after 4 weeks (Zheng et al., 2017). It's important to mention that PFOA can damaged the pancreas and induce diabetic (Margolis and Sant, 2021, Girardi and Merler, 2019). Dyslipidemia was seen directly after DM induction is due disturbance in the lipid metabolism (Abbas and Abbas, 2018). Additionally, PFOA impair protein kinase B (PKB), also known as Akt which associated with glucose metabolism and diabetic induction (Du et al., 2018).

Figure 2: Fasting blood glucose concentrations in the control, alloxan induced diabetic, treatment animals with PFOA at 100 mg/kg BW and diabetic guinea pigs exposed to PFOA at 100 mg/kg BW during the experimental period of 4 weeks. Mean ± SE, *significant differences (*p< 0.05).



Serum cholesterol concentrations

According to this study, one of the significant findings to emerge from the 2nd week of the experiment is that the cholesterol concentrations were increased in the diabetic male guinea pigs that dosed with PFOA (G4) (Figure 3-A). Moreover, the data of the 3rd week and 4th week elucidate high significant concentrations (p<0.05) of cholesterol in G2, G3 and G4 compared with control group (G1). Importantly, the results in Figure 3-A showed higher cholesterol concentrations in G4 (diabetic animals exposed to PFOA) compared with G2 (diabetic group) and G3 (PFOA treated group).

Serum triglyceride concentrations

The results displayed in Figure 3-B indicated the triglyceride concentrations in the control

(G1), alloxan induced diabetic (G2), PFOA at 100 mg/kg BW (G3) and diabetic guinea pigs exposed to PFOA at 100 mg/kg BW (G4) during the experimental period of 4 weeks. Serum triglyceride concentrations were significantly increased ($p<0.05$) in G4 that received PFOA following alloxan-induction diabetic at 1st week compared with the control G1. Furthermore, serum triglyceride concentrations were significantly raised ($p<0.05$) in G2, G3 and G4, respectively compared with control group at 2nd, 3rd and 4th weeks. Clearly, the results showed higher cholesterol levels in diabetic animals exposed to PFOA (G4) compared with diabetic group (G2) and PFOA treated group (G3), respectively.

Serum high density lipoprotein cholesterol (HDL-C)

Figure 3-C illustrated high density lipoprotein cholesterol (HDL-C) levels in the control (G1), alloxan induced diabetic (G2), PFOA at 100 mg/kg BW (G3) and diabetic guinea pigs exposed to PFOA at 100 mg/kg BW (G4) after 4 weeks. These HDL-C concentrations were significantly increased ($p<0.05$) at 2nd week in G3 that received alloxan and PFOA compared with the control group (G1). During the 3rd week, the HDL-C concentrations were significantly increased ($p<0.05$) in all treated groups (G2, G3 and G4, respectively) compared with control group. The results of the 4th week demonstrated no significant differences between control and treated groups. There was significant increased ($p<0.05$) in HDL-C in G4 compared with G1 and G2 at the 2nd and 3rd weeks (Figure 3-C).

Low density lipoprotein cholesterol (LDL-C)

Figure 3-D illustrated low density lipoprotein cholesterol (LDL-C) levels in the control (G1), alloxan induced diabetic (G2), PFOA at 100 mg/kg BW (G3) and diabetic guinea pigs exposed to PFOA at 100 mg/kg BW (G4) for 4 weeks. Low density lipoprotein cholesterol (LDL-C) concentrations were significantly increased ($p<0.05$) in G2, G3 and G4, respectively compared with control group (G1) during the 2nd, 3rd and 4th weeks of the experiment. There was a significant increase ($p<0.05$) in G4 (diabetic animals dosed with PFOA) compared with G2 (diabetic animals), respectively (Figure 3-D).

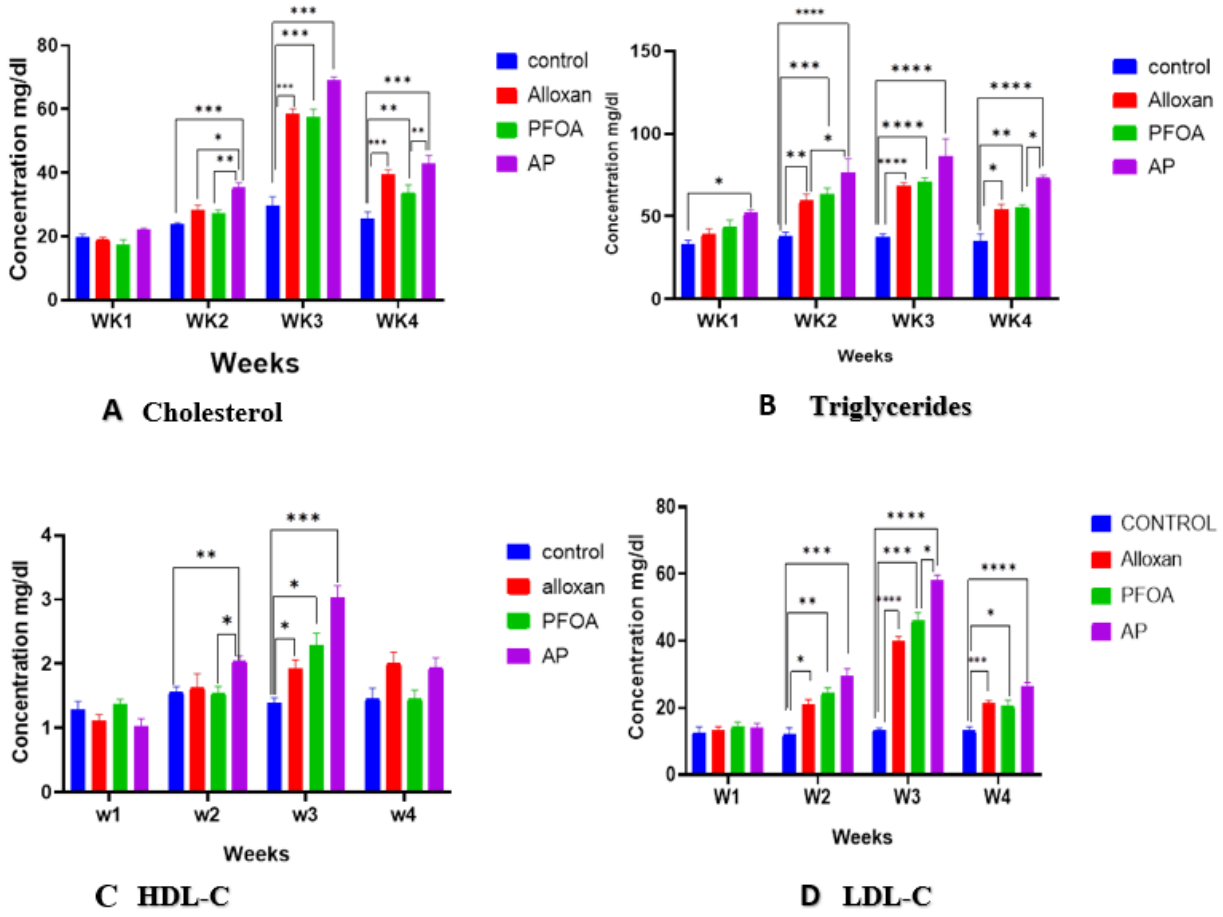
Our results showed significant increasing in the lipid profiles (serum cholesterol, serum triglyceride, serum high density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C)) after exposure to PFOA for 4 weeks (Figure 3). These results are in agreement with Frisbee et al. (2010) who revealed that PFOA exposure is associated with hyperlipidemia in human. Following the exposure to PFOA substance, an increasing blood lipid concentrations have been repeatedly detected in humans, but not in rodent studies which exhibited reverse effects (i.e. decreased blood lipid as cholesterol and triglycerides) (Fragki et al., 2021).

Earlier studies pointed that mice and rats as experimental animals are not relevant to study the changes in lipid metabolism due to differences in PPARa activation after exposure to PFOA (Fernandez and Volek, 2006). Therefore, the mechanisms that responsible for the potential PFOA induced hypercholesterolemia in humans are inverse one in rodents. In another words, PFOA reduced plasma triglycerides and cholesterol concentrations in rat and mice (depending on PPARa) while increased plasma lipid profile after long term and low dose exposure to PFOA in humans (Attema et al., 2022, Fragki et al., 2021).

Because guinea pigs have the ability to carry the majority of cholesterol in LDL, it consider as a unique model to study lipid profile and metabolism of lipoprotein that can mimic human condition (West and Fernandez, 2004). Apparently, this animal model (guinea pig) would be a useful model to further explore the toxicity of PFOA and studying their toxicity in diabetic.

Regarding PFOA toxicity assessment in human, the results of this study showed guinea pig as a promising model to study lipid profiles that mimic human exposure to PFOA.

Figure 3: (A) Cholesterol concentrations, (B) Triglyceride concentrations, (C) HDL-C concentrations and (D) LDL-C concentrations in the control (G1), alloxan induced diabetic (G2), PFOA at 100 mg/kg BW (G3) and diabetic guinea pigs exposed to PFOA at 100 mg/kg BW (G4) during the experimental period of 4 weeks. Mean ± SE, *significant differences (*p< 0.05).



Histopathological findings of liver, kidney and pancreas

For studying histopathological changes of PFOA in normal and diabetic male guinea pigs, tissues sections from liver, kidney and pancreas were examined microscopically to determine any changes following PFOA toxicity. Liver from control (G1) showed no remarkable lesions (Figure 4- A). However, animals in diabetic group showed mild degeneration of hepatocytes compared with control group

(Figure 4- B). All animals that treated with PFOA showed remarkable lesions in comparison with the control group (G1) characterized by sever distention of hepatocytes with fat vacuolation. Further, degeneration and necrosis changes were seen in the liver section (Figure 4-C). Moreover, PFOA treated diabetic group (G4) showed extensive liver damage with fatty degeneration and loss of normal liver structure. In addition, accumulated of intracytoplasmic fat droplets give rise to signet ring appearance (Figure 4-D). Perfluorooctanoic acid (PFOA) can induce liver toxicity, fatty liver (hepatic steatosis) and also effects on lipid metabolism (EPA, 2016). In addition, PFOA was reported to stimulate the accumulation of lipid droplets in the nuclei of hepatocytes (Yan et al., 2015, Sheng et al., 2016). The deposition of lipid droplets in the hepatic nuclei may have significant inferences with function of nuclear receptors and lipid signaling pathway (Barbosa and Siniossoglou, 2020). As a results, PFOA-induced lipid disturbance in the liver due to failure of specific pathway (Wu et al., 2017). In this study PFOA-treated guinea pigs showed increased number of cytoplasmic fat vesicles with signet ring nucleus and congested central vein. In addition, degenerative changes and necrosis in the liver (Figure 4, C-D).

The histological sections of the kidney of control group had no noticeable lesion (Figure 5- A).

Kidney sections that treated with alloxan (G2) showed dilation of Bowman's space with degeneration of renal tubular epithelium and atrophy of glomerular tuft (Figure 5-B). Treated animals with PFOA (100 mg/kg BW) (G3) showed necrotic changes in the lining tubular epithelium with sloughing of some epithelia and occluded the lumen (Figure 5-C). Furthermore, kidney sections of diabetic male guinea pigs exposed to PFOA (G4) showed degeneration and necrosis of tubular epithelium with atrophy of glomerular tuft, dilatation of Bowman space and mononuclear cell infiltration (Figure 5-D). The toxic effects of PFOA were related to the kidney dysfunction at different doses of oral exposure in mice for 10 days study (Rashid et al., 2020). Necrosis of tubular epithelial cells with sloughing, degeneration of some epithelia and occluded the lumen in G3 and G4, respectively (Figure 5, C-D) agreed with Mohammed et al. (2014) who reported that PFOA caused necrotic alterations in epithelial lining of renal tubules with accumulation of eosinophilic substance in tubular lumen with irreversible changes involving the proximal tubules at lower dose with glomerular alteration at high doses.

Histopathological sections of pancreas of control group (G1) showed normal histology of pancreatic acini and islets of Langerhans with normal interlobular duct (Figure 6-A). In diabetic group (G2) pancreas showed vacuolation and necrosis of Langerhans islets with congested blood vessels (Figure 6-B). Histopathological sections of pancreas in G3 (animals dosed with PFOA) showed pancreatic cell hyperplasia with blood vessels congestion (Figure 6-C). In diabetic male guinea pigs dosed with PFOA (G4), pancreas showed necrosis and coalescing of Langerhans islet with prominent islet cell hyperplasia (Figure 6-D). Figure-7 (A-B) showed intraductal papillary growth, mucinous epithelium proliferation with goblet cell hyperplasia.

The pancreas of guinea pigs characterized by the presence of only one minor pancreatic duct that different from most rodents which have major pancreatic duct (Al-Saffar and Nasif, 2020). Alterations in the cytoarchitecture of the pancreas with increased serum insulin level and reduce glucagon content were recorded in mice

following PFOA exposure (Wu et al., 2017). The results of the current study are in accordance with (Kamendulis et al., 2014) who related the histopathological changes (focal ductal hyperplasia) in the pancreas of mice treated with PFOA with increased of the oxidative stress.

These results suggested that pancreas is a target organ for PFOA toxicity that responsible for stimulation an inflammatory response. Further, chronic exposure to PFOA has been reported to develop pancreatic acinar cell tumors in rat (Lau et al., 2007) and in human (Kamendulis et al., 2022). Moreover, reactive oxygen species (ROS) plays an important role in PFOA-induced toxicity in pancreas and incidence of inflammation following PFOA exposure (Abudayyak et al., 2021). Previous reports suggested that PFOA leads to decrease the activity of glutathione reductase (GR) (Zhang et al., 2019) and significant increase in the oxidative stress (Zhang et al., 2021).

Figure 4: Histopathological sections of liver from guinea pigs at the end of the experimental period of 4 weeks. (A): control group (G1) shows normal liver architecture. (B): alloxan induced diabetic (G2) shows mild cytoplasmic degeneration (arrow head) (C): treated animal with PFOA at 100 mg/kg BW PFOA (G3) shows sever destruction of hepatocytes with a large vacuole (arrow head) and necrosis (circle). (D) diabetic guinea pigs exposed to PFOA at 100 mg/kg BW (G4) shows extensive liver damage with macrovesiculor degeneration, large fat vacuole and peripheral location nucleus (signet ring cell appearance) (arrow head). H & E stains, (X40).

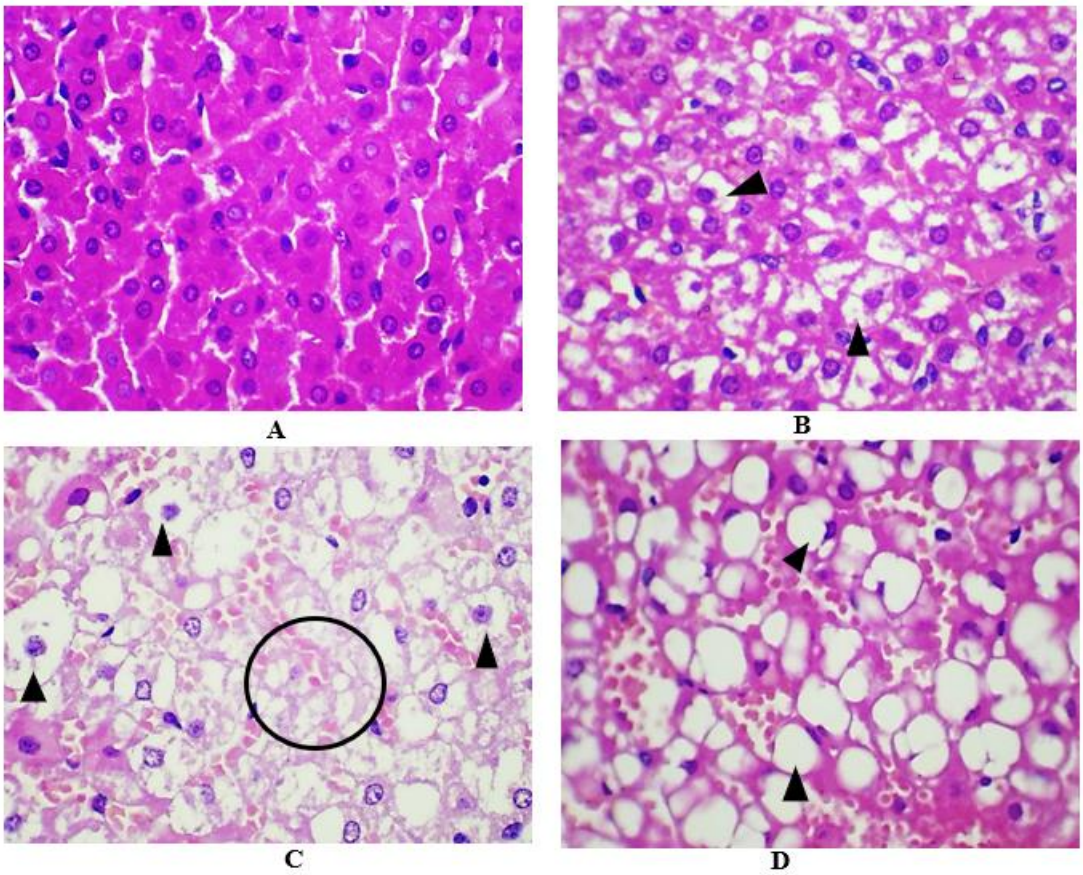


Figure 5: Histopathological sections of kidney from guinea pigs at the end of the experiment period of 4 weeks. (A) control group (G1) shows normal histological appearance (X10). (B) alloxan induced diabetic (G2) shows dilation of Bowman's space (arrow) and degeneration of renal tubules (arrow head) (X20). (C) treated animal with PFOA (100 mg/kg BW) (G3) shows necrotic changes in the lining tubular epithelium (arrow head) with fragmented nuclei (arrow) and sever destruction of glomeruli (X40). (D) diabetic guinea pigs exposed to PFOA at 100 mg/kg BW (G4) shows mononuclear cell infiltration (arrow) and atrophy of glomerular tuft (arrow head) (X20), (H&E stains).

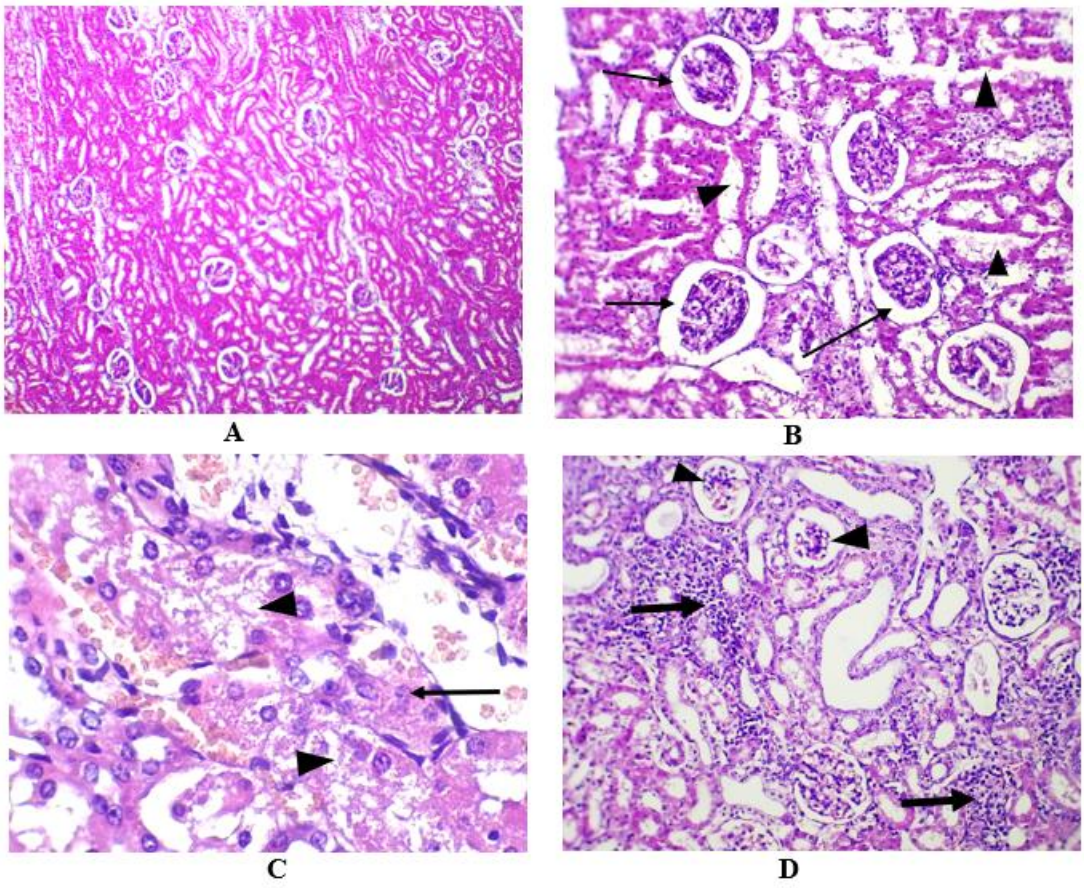


Figure 6: Histopathological sections of pancreas from guinea pigs at the end of the experiment period of 4 weeks. (A) control group (G1) shows normal structure of pancreatic islets of Langerhans (arrow) with normal lipid contents (arrowhead) and interlobular septa (star), X10. (B) alloxan induced diabetic (G2) shows vacuolated and necrosis of Langerhans islets (arrow) and congested blood vessels (arrowhead), X20. (C) treated animal with PFOA (G3) shows pancreatic cell hyperplasia with blood vessels congestion (arrow), X40. (D) diabetic guinea pigs treated with PFOA 100 mg/kg (G4) shows coalescing Langerhans islet with prominent islet cell hyperplasia (arrow) X20, (H&E stain).

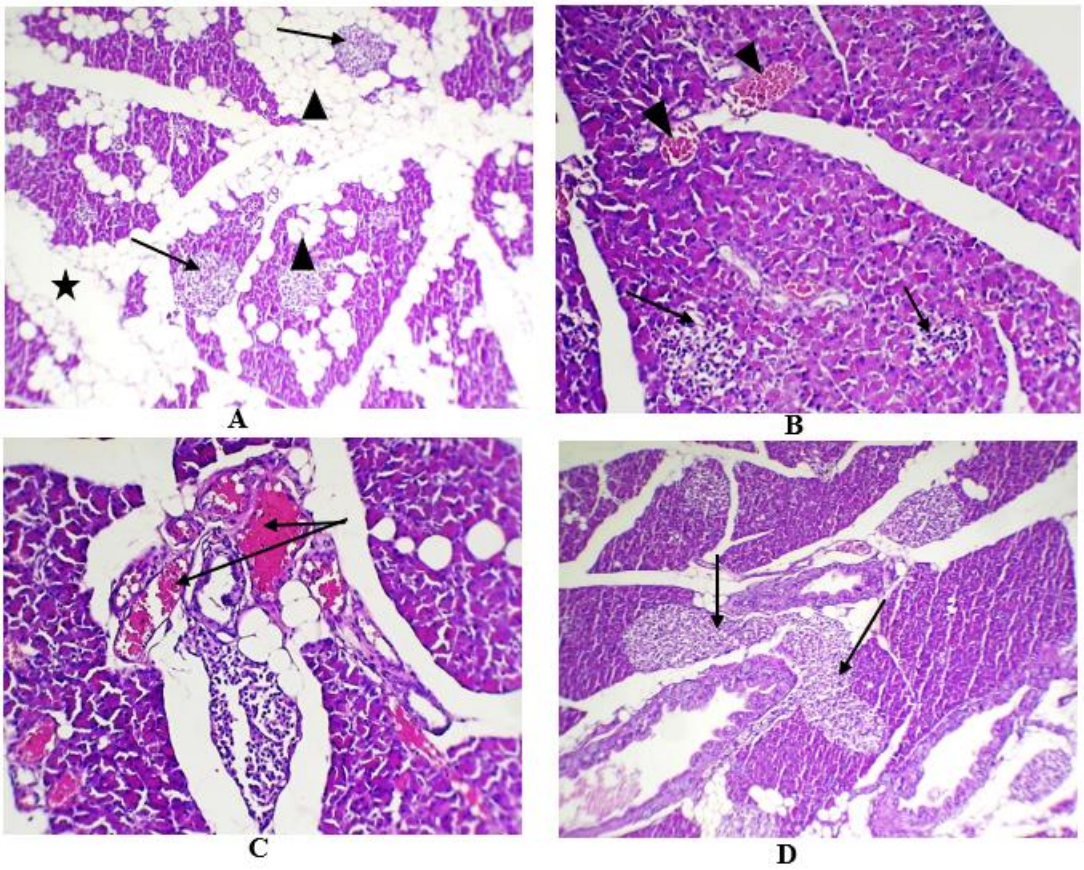
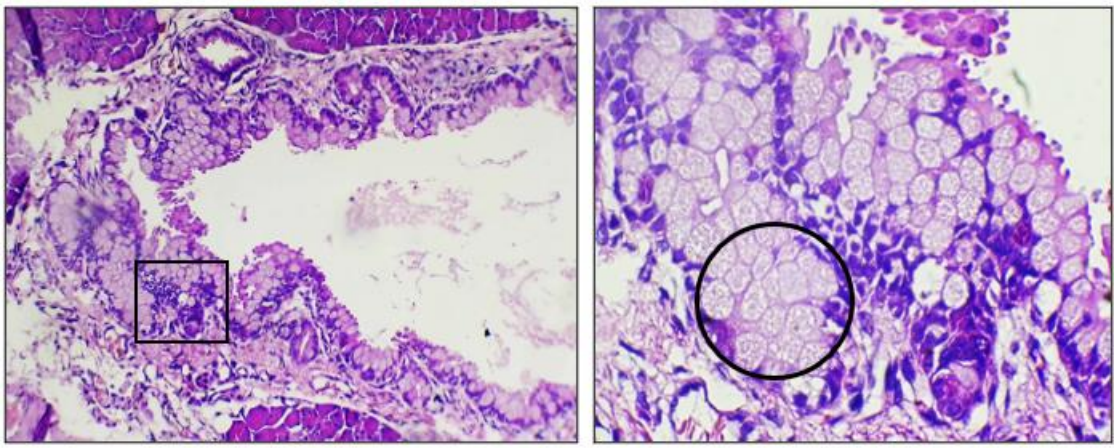


Figure 7: Histopathological sections of pancreas from diabetic male guinea pig treated with PFOA 100 mg/kg BW for 4 weeks (G4). (A): shows intraductal papillary growth (square), mucinous epithelium proliferation (20X); (B): At higher magnification of (A) shows goblet cell hyperplasia (circle) (40X) (H&E stain).



Conclusion

Developing an animal model to study the association changes in lipid profiles and glucose levels correlated with PFOA exposure is a powerful tool to better understand the toxicity of this substance in human. Similar to humans a positive association of PFOA with serum cholesterol and glucose was detected using guinea pig. The observation here provided insight that the guinea pig may serve as an alternative model for the evaluation of PFOA toxicity in human rather than the reverse effect in mice and rat.

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