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Screening the Toxic effect of Polyethylene Terephthalate Nanoplastics on Kidney of Adult Male Swiss Albino Mice with Promising Betaine Alleviation

Nehal A. Kamel[1*], Dina W. Bashir[1], Ebtihal M.M. EL-Leithy[1], Adel. F. Tohamy[2], Maha M. Rashad[3], Ghada E. Ali[3] and El-Saba A.A.[1]

[1]Cytology and Histology Department, Faculty of Veterinary Medicine, Cairo University, Egypt
[2]Toxicology and Forensic Medicine Department, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt
[3]Biochemistry and Molecular biology Department, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt
***Corresponding Author:** Nehal A. Kamel, **E-Mail:** nehalalsayed@cu.edu.eg

ABSTRACT

Polyethylene terephthalate nanoplastics (PET-NPs) are utilized in the production of medical bionic materials and the packaging of beverages. Betaine is a ubiquitous natural constituent present in organisms such as plants, animals, and microorganisms. So, the current investigation tries to find out if PET-NPs could seriously harm mice's kidneys and whether betaine could have any ameliorative effects. In this study, a total of 40 mice were separated into four groups (ten mice in each): Group I (performed as the control group), Group II (received 1000 mg/kg betaine intraperitoneally), Group III (received 200 mg/kg PET-NPs orally), and Group IV (was given betaine first, and after 1 hour, PET-NPs were given at dosages that were the same as those given to groups II and III, respectively) daily for a month. Serum and kidney samples were collected and processed for biochemical and histological assessments. The current study found that PET-NPs significantly increased blood urea nitrogen (BUN), creatinine, and malondialdehyde levels (MDA), while reducing glutathione (GSH) levels. The histological examination revealed multiple histopathological alterations. The PET-NPs-exposed group demonstrated renal corpuscle hypotrophy, a loss of cellular structure in some proximal convoluted tubules (PCT) and distal convoluted tubules (DCT). The renal medulla exhibits hyalinization, congestion, and degeneration of collecting tubules. Conversely, the pre-administration of betaine results in a decline in BUN, creatinine, and MDA concentrations. Furthermore, there is a rise in GSH levels and the group pretreated with betaine showed significant improvement in kidney architecture, with the renal cortex showing almost normal architecture and the collecting tubules in the renal medulla slightly improving. In conclusion, betaine showed a promising nephroprotective effect against PET-NP-induced toxicity.

Keywords: BUN, Creatinine, Kidneys, Oxidative stress, Polyethylene terephthalate nanoplastics.

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INTRODUCTION

Plastic waste poses a persistent environmental threat due to its growing consumption and limited recycling capabilities **(Mitrano *et al.*, 2021)**. Concerns have grown about the buildup of microplastics and nanoplastics **(Gerdes *et al.*, 2019)** which can be derived from degradation**(Lambert and Wagner, 2016)** or manufactured for various applications, including personal hygiene products, pharmaceuticals, and industrial use **(Fendall and Sewell, 2009; Ambrogi *et al.*, 2017 and Ishmukhametov *et al.*, 2022)**. Plastic NPs can be directly or indirectly absorbed by aquatic and terrestrial organisms **(Moore, 2006; Smith *et al.*, 2018)**, with potential health implications in the human food supply chain **(Committee *et al.*, 2018)**.

Polyethylene terephthalate nanoplastics (PET-NPs), commonly used in food and beverage receptacles **(Haldar *et al.*, 2023)** should be thoroughly examined for potential health risks. Interestingly, NPs were shown to accumulate in the kidney following oral administration **(Deng *et al.*, 2017)**. Multiple investigations have shown evidence that NPs can induce genotoxicity, mutagenicity and immunotoxicity **(Elizalde-Velázquez *et al.*, 2020; Guimarães *et al.*, 2021)**. Moreover, PET-NPs can induce nephrotoxicity as well as trigger inflammation **(Lin *et al.*, 2023)**.

Betaine, a choline-derived compound is synthesized by the liver and is beneficial for dietary intake from vegetables. It acts as an organic osmolyte providing cellular protection against stress **(Eklund *et al.*, 2005; Lipinski *et al.*, 2012).** Furthermore, it serves as a catabolic reservoir of methyl groups, commonly referred to as methyl donors **(Craig, 2004)**. Betaine's ability to withstand oxidative stress **(Ozturk *et al.*, 2003)** protect the kidney from harmful chemicals **(Hagar and Al Malki, 2014)** and act as an anti-inflammatory peptide and antioxidant has been proven in various studies. Hence, the impact of betaine on oxidative stress may have a substantial effect on its ability to protect cells **(Ommati *et al.*, 2021)**, also reduce apoptosis **(Khodayar *et al.*, 2018)**.

To our knowledge, the adverse effects of PET-NPs have not been well investigated yet. Thus, the goal of our work was to assess the nephrotoxic effects of PET-NPs on mice and then evaluate the possibility of betaine being able to mitigate this toxicity by leveraging its antioxidant and anti-inflammatory characteristics.

MATERIALS AND METHODS

1. Chemicals

The Betaine $C_5H_{11}NO_2$ was supplied by El-Mekkawy Company, located in Cairo, Egypt, to the Amargain industrial complex situated in Khopat Thane, Mumbai, Maharashtra, India. The NPs were synthesized by the National Research Centre, located at 33 El Bohouth St. 12622, Dokki, Giza, 12311, Egypt. All reagents and chemical compounds used in the experiment were of exceptional quality.

2. Characterization of PET-NPs

2.1 PET-NPs preparation

The precipitation procedures outlined in a prior investigation **(Rodríguez-Hernández *et al.*, 2019)** were employed for the synthesis of PET-NPs. At first, 10 grams of PET were mixed with 100 milliliters of 90% trifluoroacetic acid (TFA). This process was carried out for duration of 2 hours under conditions of intense stirring. The dissolving process took place in a sealed, opaque vessel so that the TFA would not evaporate or break down. After complete dissolution, a volume of 100 ml of TFA (20%) was gradually added drop by drop to the precipitate. This process was repeated three times to guarantee thorough mixing of the PET nanoparticles. Subsequently, the precipitate was subjected to three washes in order to effectively remove any remaining TFA. The precipitation of PET nano was completed using a centrifugation process operating at a speed of 3000 rpm. The solution used was 100 ml of water with 0.5 g of sodium dodecyl sulphate (SDS). This was done to make a milky nanoemulsion of PET. Following a 15-minute period of stirring, the solution underwent 5 minutes of sonication.

2.2 Transmission electron microscopy (TEM) of PET-NPs

The morphology of the PET nano-emulsion was examined using a transmission electron microscope (TEM, JOEOL Co., JEM-2100, Tokyo, Japan) operating at room temperature. High-tension electricity at 160 kV was utilized for this purpose. The photographs were captured using high-magnification techniques to investigate the precise shape and morphology of the PET-NPs. We used the Gaussian fitting in the histogram tells the average particle size.

3. Experimental animals and ethical approval

Forty mature male Swiss albino mice, each weighing approximately 30 ±2 grams, were acquired from the VACSERA animal institution in Egypt. The experimental animals underwent a two-week acclimation period before the experiments started. The animals were housed in polypropylene mouse cages with dimensions of 45 cm (length) x 45 cm (width) x 25 cm (height). The cages were maintained at a temperature of 22 ± 2 °C and a relative humidity of 50% in a controlled laboratory environment. There was an established cycle of 12 hours of light and 12 hours of darkness. Every cage housed a maximum of five animals and had stainless steel upper grills, along with soft wood sawdust used as bedding material. They possessed a surplus of food and beverages. All the animals received compassionate treatment and care. The experimental methodologies employed in this study have received approval from the Institutional Animal Care and Use Committee (IACUC) of Cairo University's Faculty of Veterinary Medicine, under Protocol number Vet CU 08072023701.

4. Experimental design

Following the completion of the adaptation period, the animals were randomly divided equally into four groups. Each group consisted of ten mice (n

= 5 per cage) and was determined based on the trial. The trial was administered on a daily basis for duration of 30 days. The study involved four distinct groups: group I, untreated negative control and received only water; group II, the betaine-treated group, in which mice were intraperitoneally administered betaine alone at a dose of 1000 mg/kg b.w. **(Khodayar *et al.*, 2018)**; group III, the PET-NPs-treated group, in which mice were orally administered PET-NPs in dosage of 200 mg/kg b.w. using a gavage needle **(Lin *et al.*, 2023)** and in group IV, the betaine plus PET-NPs-treated group, mice were first given 1000 mg/kg b.w. of betaine intraperitoneally. After an hour, they were given 200 mg/kg b.w. of PET-NPs orally.

5. Sample collection and preparation

After duration of 30 days, all animals were sedated, and blood samples were collected from the inner corner of the eye of each Swiss albino mouse. The blood samples were obtained using capillary tubes and then centrifuged at a speed of 3,000 rpm for duration of 20 minutes. The resultant serum samples were preserved at a temperature of -20 ℃ for the assessment of renal functioning. Then cervical dislocation within a period of 30 minutes. Kidney specimens were cryopreserved at a temperature of -80 ℃ to facilitate their investigation with the aim of evaluating oxidative stress characteristics. To perform a histological investigation, we fixed additional specimens using 10% neutral buffered formalin (NBF).

6. Biochemical examination

6.1. Determination of kidney function biomarkers

The levels of blood urea nitrogen (BUN) and creatinine were assessed using commercial reagent kits following the provided instructions (Bio-diagnostic Co., Giza, Egypt).

6.2. Determination of renal oxidative stress biomarkers

The kidney samples were evaluated for markers of oxidative stress, specifically glutathione (GSH) and lipid peroxidation biomarker (LPO) malondialdehyde (MDA). The assessment was conducted following the methods described by **Beutler *et al.*, (1963) and Satoh (1978)**, respectively. Commercially available kits from Bio-diagnostics were used for this purpose. In summary, the kidneys were subjected to perfusion using a phosphate-buffered saline solution with a pH of 7.4, which was supplemented with 0.16 mg/ml of heparin. This perfusion process aimed to eliminate any presence of red blood cells and clots within the kidneys. The tissue samples were homogenized in a cold buffer solution consisting of 50 mM potassium phosphate and 1 mM EDTA, with a volume of 5–10 ml per gram of tissue. Homogenization was performed using a tissue homogenizer. Subsequently, the homogenate was subjected to centrifugation at 4,000 revolutions per minute for a duration of 15 minutes at a temperature of 4°C. The supernatant was stored at a temperature of -80°C and measured using a spectrophotometer.

7. Histopathological examination (Light microscope)

The renal samples were properly dissected and preserved in a 10% NBF for 48 hours. The tissue was washed, submerged in increasing concentrations of ethanol to remove moisture, and subsequently treated with xylene for clarification. Afterwards, they were immersed in paraffin wax, cut into slices that were 3–4 μm thick, processed to remove the paraffin, and finally stained with hematoxylin and eosin (HandE)**(Bancroft *et al.*, 2013)**.

The histological alterations were evaluated via a conventional semiquantitative scoring technique to determine the extent of severity among several groups. Each group consisted of five slides, each representing the kidneys of five mice. In the absence of any abnormalities in histology (0), mild (1), moderate (2), severe (3), and extensive severe (4) tissue damage is indicated, with corresponding percentages of less than 25%, 25-50%, 50-75%, and greater than 75%, respectively **(Khalaf *et al.*, 2021)**. The main histopathological signs employed to assess renal injury encompass glomerular atrophy, tubular degeneration, congestion, hyalinization, and inflammatory cells infiltrations.

8. Statistical analysis

With the exception of the histopathological scoring data, which were nonparametric and expressed as median ± interquartile range, all other data were parametric and presented as mean ± standard error (SE). These data were analyzed using SPSS version 28 software (IBM, USA) through ANOVA followed by a least significant difference post hoc test. The Kruskal-Wallis test and the Mann-Whitney U test were both used to assess the data on histopathological lesion scores. The concept of statistical significance was operationalized as a p-value of 0.05.

RESULTS

1. Morphology of the prepared PET-NPs

The PET-NPs utilized in our experiment underwent comprehensive characterization. In general, the nanoplastics exhibited a mostly spherical form and demonstrated excellent dispersion under TEM andthe Gaussian fitting in the histogram tells the average particle size = 96.265 nm (**Fig.1)**.

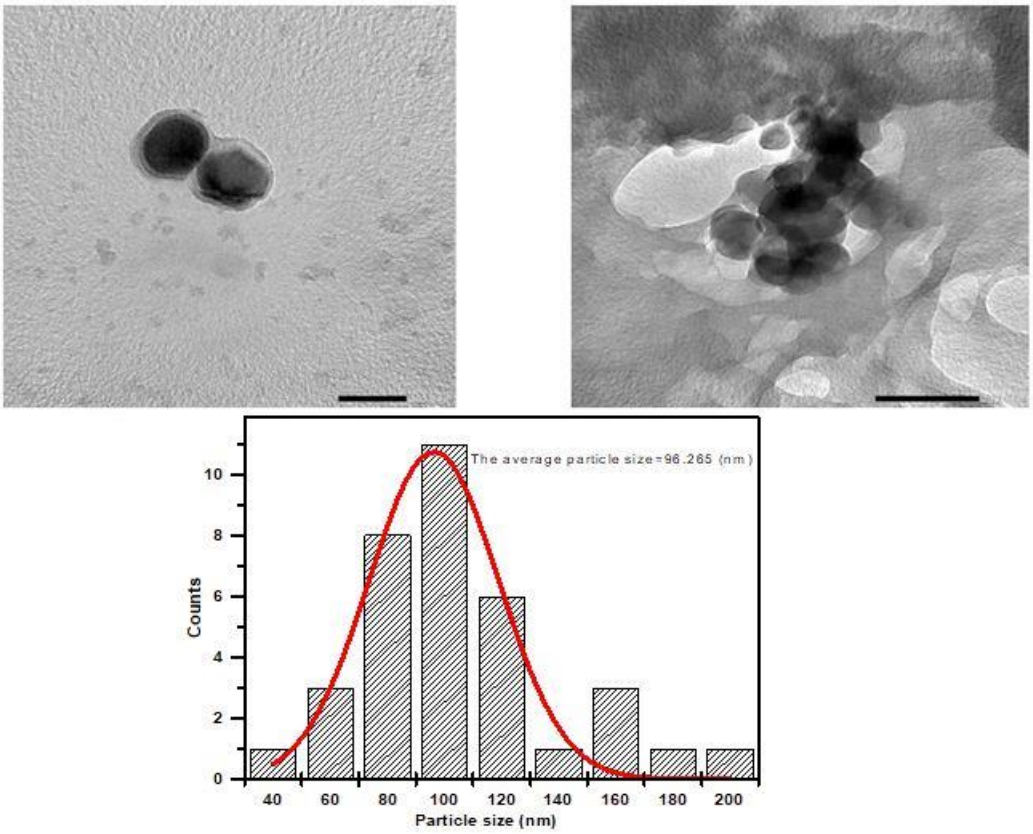

Fig.1: Morphology of PET-NPs by TEM

2. Biochemical investigations

2.1. Effect of PET-NPs on kidney function

The mice that orally received PET-NPs exhibited a significant elevation in the concentrations of creatinine and BUN as compared to the control group. Co-administration of betaine resulted in a substantial reduction in the concentrations of creatinine and BUN, as depicted in **Fig. 2 and 3**, when compared to the corresponding PET-NPs ($p < 0.05$).

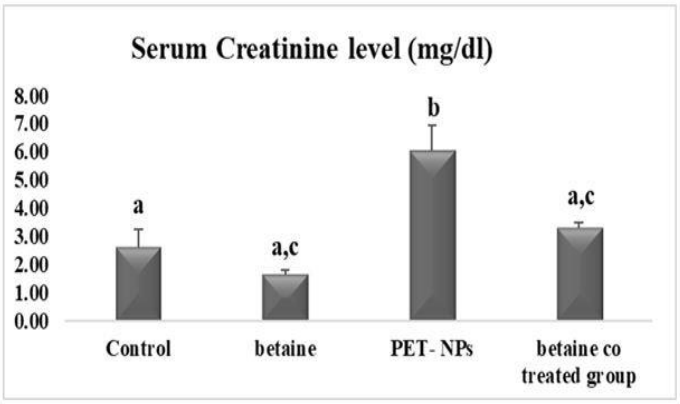

Fig. 2: Showing creatinine level (mg/dl) in different groups (n=5)

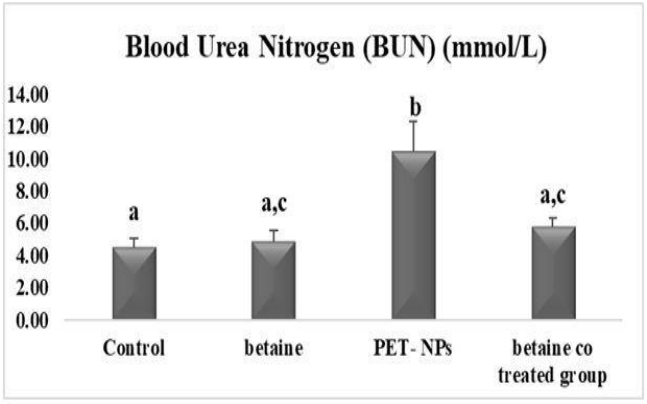

Fig.3: Demonstrating BUN level (mmol/L) in different groups (n=5)

Data are represented as Mean ± SEM.

[(a)] significantly different from corresponding PET-NPs group at $P \leq 0.05$.

[(b)] Significantly different from corresponding control group at $P \leq 0.05$.

2.2. Effect of PET-NPs on oxidative stress biomarkers (MDA and GSH)

The experimental group of mice that were exposed to PET-NPs exhibited a substantial reduction in the level of GSH and an elevation in the level of lipid peroxidation, as expressed by the concentration of MDA, when compared to the control group. This difference was statistically significant at a significance level of $p < 0.05$. The co-administration of betaine in mice demonstrated an important rise in kidney production against oxidative stress caused by PET-NPs. This was evidenced by a substantial elevation in the GSH level and a considerable reduction in the level of MDA, as illustrated in **Figs. 4 and 5**.

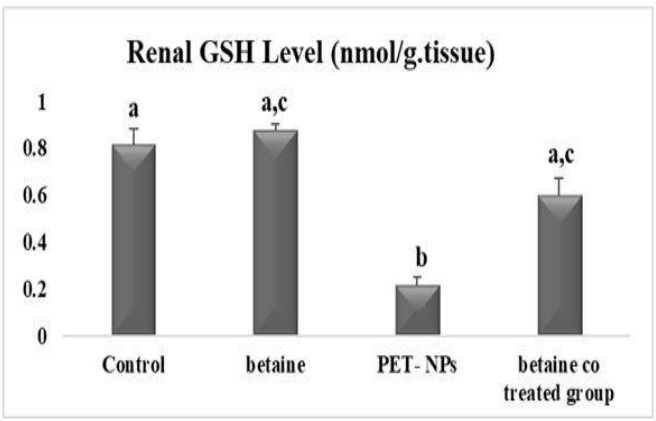

Fig.4: Representing GSH level (mmol/g. tissue) of kidney in different groups (n=5).

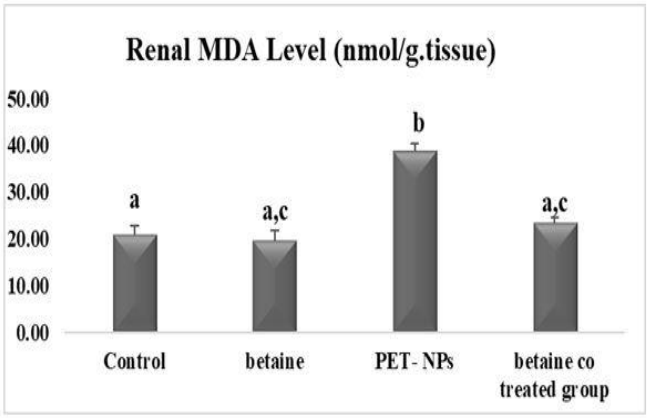

Fig.5: Illustrating MDA level (nmol/g. tissue) of kidney in different groups (n=5).

Data are represented as Mean ± SEM.

[(a)] significantly different from corresponding PET-NPs group at $P \leq 0.05$.

[(b)] Significantly different from corresponding control group at $P \leq 0.05$.

3. Histopathological examination

The evaluation of kidney slides stained with HandE in the control group (Group I) and the betaine group (Group II) of adult male Swiss albino mice revealed the presence of typical histological features, including the renal cortex and renal medulla. The renal cortex had renal corpuscles of typical size, which contained glomerular capillaries and were surrounded by Bowman's capsules. The proximal convoluted tubules (PCT) were lined with pyramidal cells and had narrow lumina, while the distal convoluted tubules (DCT) were lined with cuboidal cells and had wide lumina (Fig. 6 **a, b)**. The renal medulla is composed of collecting tubules (CT) that are lined by cuboidal epithelial cells and interstitial blood capillaries (Fig. 6 **c,d)**.

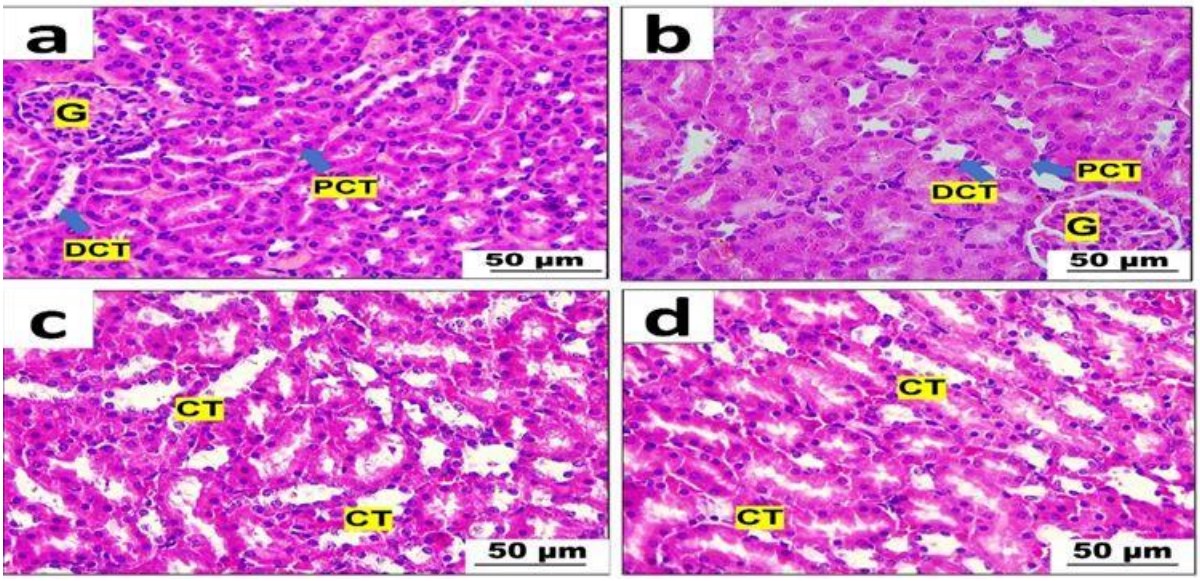

Fig.6: Renal tissue sections from adult male Swiss albino mice at HandE X400. **(a & c)** Control mice (group I) and **(b & d)** Betaine treated group (group II) demonstrating **(a &b)** Renal cortex revealing normal histological structure, glomerulus (G) containing glomerular capillaries, proximal convoluted tubule (pct), and distal convoluted tubule (dct). **(c& d)** Renal medulla containing collecting tubules (CT) lined by cuboidal cells.

The renal cortex of the PET-NPs-exposed group revealed a variety of histopathological changes in the renal corpuscles, glomeruli, PCT, DCT, and renal vasculature **(Fig.7a–e)**. Some renal corpuscles exhibited a narrowing in the capsular space and pyknosis in the nuclei of intraglomerular cells **(Fig.7a)**. Moreover, certain renal corpuscles exhibited hypotrophy and degeneration, accompanied by a loss of content **(Fig.7b)**. Furthermore, the presence of pronounced vascular congestion, hyalinization, and migration of inflammatory cells into the renal cortex were detected (**Fig.7c)**. Additionally, certain PCT and DCT exhibited cellular architectural deterioration, characterized by pyknosis of their nuclei with flattening and some shedding of cellular cytoplasm as strands within the tubular lumina. In others, there was a complete absence of cytoplasmic acidophilia in the lining cells of the DCT **(Fig. 7a, c)**, while other regions of the DCT displayed vacuolization **(Fig. 7d)**. Moreover, the renal medulla sections exhibited interstitial haemorrhage, hyalinization, and congestion of interstitial blood vessels, as described in **(Fig.7e)**. Some of the collecting tubules exhibited degeneration characterized by the loss of cellular architecture. Additionally, there was evidence of cytoplasmic acidophilia diminution and shedding of cytoplasmic content. Furthermore, pyknosis and flattening of certain nuclei were observed **(Fig. 7e, f)**.

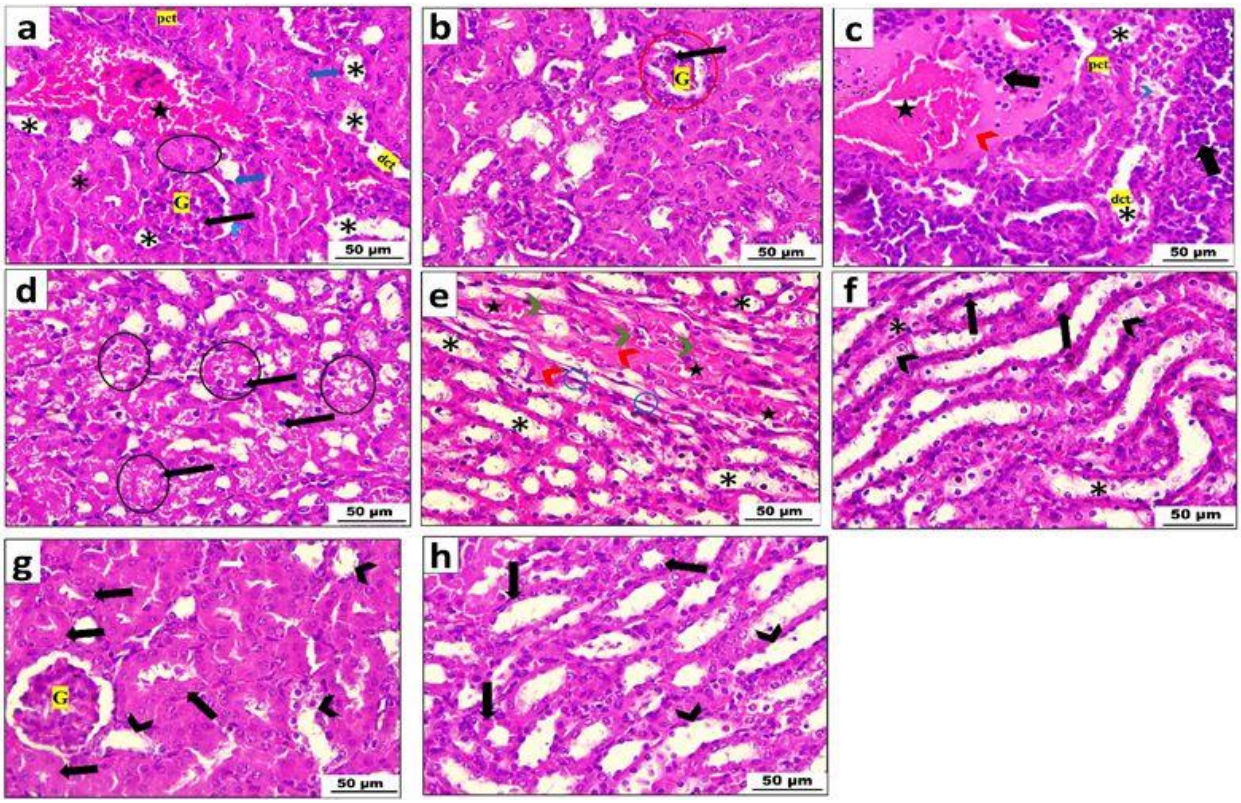

Fig. 7: A photomicrograph of HandE-stained sections of mice' renal tissues at X400 shows: **a-f**: The PET-NPs-exposed group had various histopathological changes. **a**: Some renal corpuscles (G) show narrowing of capsular space (blue arrowhead) and pyknosis in the nuclei of intraglomerular cells (black arrow); vascular congestion (star) is observed; and degeneration of PCT and DCT (asterisk) with flattening of some nuclei (blue arrow) and complete loss of cytoplasmic acidophilia in some cells (circle). **b**: Some glomeruli (G) show hypotrophy (red circle) with loss content (arrow) as observed. **c**: Vascular congestion (star) with hyalinization (red arrowhead) and inflammatory cell infiltrations (black arrow). Also, some degenerated PCT and DCT (asterisk) and the shedding of cellular cytoplasm as strands within the tubular lumen (blue arrowhead). **d**: Some DCT show vacuolation (black circle) with pyknotic nuclei (black arrow). **e-f**: Renal medulla of the PET-NPs-exposed group **e**: shows interstitial haemorrhage (green arrowhead), hyalinization (red arrowhead), and congestion of interstitial blood capillaries (black star). In addition, collecting tubules were degenerated (asterisk) with a flattened epithelial lining (blue circle). **f**: Collecting tubules were degenerated (asterisk) with pyknotic nuclei (black arrow) with shedding of cellular cytoplasm as strands within the tubular lumen (black arrowhead). **g-h**: The betaine-cotreated group **g**. shows restoration of the normal structure of most renal corpuscles, glomeruli (G), and renal tubules (black arrow), while some renal tubules still show degeneration (asterisk). **h**: A partial improvement of the renal medulla with most of collecting tubules appeared nearly normal (black arrow). Also, some tubules appeared degenerated (black arrowhead).

On the contrary, the group that received betaine co-treatment exhibited an obvious enhancement in the renal architecture. The renal cortex exhibited almost normal architecture with minimal degeneration, congestion, and hyalinization, as depicted in **(Fig.7g).** A partial improvement in the renal medulla, characterized by most of collecting tubules appearing nearly normal with a few still deteriorating **(Fig. 7h)**. The scoring of the histopathological lesions significantly illustrated the nephroprotective effect of betaine against PET-NPs-induced toxicity **(Table 1).**

Table 1: Histopathological scoring criteria for renal architecture alterations. PET-NPs-exposed group scored highest, while betaine-co-treated group showed decreased score.

Histopathological lesions	Control group	Betaine group	PET-NPs exposed group	Betaine co-treated group
Glomerular atrophy	0 ± 0^{a}	0 ± 0^{a}	3 ± 1^{b}	1 ± 1^{ac}
Tubular degeneration	0 ± 0^{a}	0 ± 0^{a}	3 ± 1^{b}	1 ± 1^{c}
Congestion	0 ± 0^{a}	0 ± 0^{a}	4 ± 2^{b}	1 ± 1^{c}
Hyalinization	0 ± 0^{a}	0 ± 0^{a}	3 ± 1^{b}	1 ± 1^{c}
Inflammatory cell infiltrations	0 ± 0^{a}	0 ± 0^{a}	3 ± 2^{b}	1 ± 1^{c}

DISCUSSION

In recent times, NPs have been discovered to pollute several environmental systems, such as marine and freshwater ecosystems, as well as the atmosphere and soil systems. **Della Torre *et al.*, (2014); Dris *et al.*, (2016); Alimi *et al.*, (2018), and Wahl *et al.*, (2021)** have all provided documentation of the aforementioned findings. As emphasized by **Wang *et al.*, (2021)**, NPs have the ability to enter the human body by inhalation, skin absorption, and oral ingestion. Moreover, an increasing number of studies have consistently shown that NPs can induce physiological toxicity in both freshwater and marine organisms. Nevertheless, there is a lack of comprehensive research on the physiological impacts of NPs on humans and other mammals **(Xu *et al.*, 2021)**. According to a recent study, NPs have the capacity to penetrate many tissues and cause significant bioaccumulation, leading to increased levels of toxicity **(Xu *et al.*, 2022)**. The presence of NPs can cause harmful changes at the cellular level, including various effects such as immediate cell death, inflammation, and damage to DNA **(Nel *et al.*, 2006)**. This study used PET-NPs to see how harmful they were to the kidneys of adult Swiss albino mice and to look into whether betaine could have a positive effect.

Our study revealed that PET-NPs can potentially lead to nephrotoxicity in certain circumstances. A significant increase in kidney biomarkers and a rise in reactive oxygen species (ROS) production support this. An increase in creatinine and BUN levels is evidence that PET-NPs have damaged the kidneys. This was further confirmed through a histopathological investigation. These findings are consistent with the findings reported by **Amereh *et al.*, (2019) and Ahmed *et al.*, (2022)**. Creatinine is an important diagnostic marker of renal function and a highly dependable indicator of impaired glomerular filtration rate. BUN is commonly considered a key indicator of renal dysfunction, as it shows an elevation in concentration after any type of kidney injury **(Ferguson and Waikar, 2012)**. The changes occurring in the glomeruli and renal tubules of the kidney may be responsible for the increase in biomarkers of kidney function. When kidney damage occurs, proximal cells release biomarkers into the bloodstream. Therefore, the elevated levels of these biomarkers at higher altitudes suggest a decline in PCT **(Zhu and Cao, 2012)**. However, the intraperitoneal injection of 1000 mg/kg of betaine effectively corrected the mentioned abnormalities in this study. The findings presented in this study are in line with the existing literature, as evidenced by earlier studies conducted by **Hagar and Al Malki (2014) and Al Za'abi *et al.*, (2021)**.

The renal tissue is more susceptible to oxidative damage because of its high concentration of long-chain polyunsaturated fatty acids **(Yin *et al.*, 2019)**. New research has strong evidence that there is a significant link between being exposed to NPs and oxidative stress **(Li *et al.*, 2020a; Li *et al.*, 2022)** which leads to DNA damage **(Prüst *et al.*, 2020; Sarasamma *et al.*, 2020)**. Furthermore, it has been noticed that oxidative stress is essential in triggering the inflammatory response by increasing the production of pro-inflammatory cytokines and

promoting the proliferation of inflammatory cells in tissues **(Banerjee and Shelver, 2021)**. Furthermore, it has been noted that the persistent inflammatory reaction could lead to heightened oxidative stress and a decline in cellular antioxidant defences **(Joffre and Hellman, 2021)**.

The current study examined the levels of MDA and GSH to assess the response of renal tissue to oxidative stress induced by PET-NPs. The MDA levels in the renal homogenate were significantly higher compared to the control group. According to statistical analysis, the mice who received treatment with PET-NPs showed a significant decrease in GSH levels. MDA is being utilized as an indicator of lipid peroxidation, and researchers have discovered a correlation between its concentration and oxidative reactions in different tissues of the body. Several recent studies have demonstrated a connection between increased MDA levels and kidney damage in various experimental conditions **(Al Asmari *et al.*, 2017; Shi et *al.*, 2017)**. It is widely recognized that GSH plays a crucial role as a cellular antioxidant, effectively combating and eliminating various ROS **(Aziz *et al.*, 2019)**. This emphasizes the importance of free radicals in the occurrence of oxidative cellular damage resulting from the harmful impacts of PET-NPs. According to **Kim *et al.*, (2013)**, LPO is a well-known indicator of oxidative stress-related cellular damage. In recent studies conducted by **Rubio *et al.* (2020) and Li *et al.*, (2020b)**, it was found that NPs can trigger the production of intracellular ROS and lead to primary DNA damage. In situations where there are excessive amounts of free radicals, the human body utilizes its own internal cell antioxidants, both enzymatic and nonenzymatic, to safeguard itself. Antioxidants play a crucial role in safeguarding against the detrimental effects caused by free radicals **(Hu and Palić, 2020)**.

Our study involved a thorough examination of the impact of PET-NPs on the kidneys of male adult Swiss albino mice, with a specific focus on observing any histopathological changes in this organ. Comparing tissue sections from adult male Swiss albino mice that were given 200 mg/kg of PET-NPs to those that were not exposed to the particles showed different histological changes. In particular, some renal corpuscles showed signs of hypotrophy and degeneration, leading to the loss of their contents. This finding is consistent with the study by **Ahmed *et al.*, (2022)**. According to a study conducted by **Nashwa and Samira (2010)**, the histological changes seen in renal corpuscles may point to a potential compromise in renal function. The PCT and DCT showed many important changes, including the disruption of cellular structure and the buildup of eosinophilic material in the tubules' lumen. **Abdelhalim and Jarrar (2011); Ibrahim *et al.*, (2018), and Ahmed *et al.*, (2022)** proposed the aforementioned observations. In addition, DCT has identified the release of cellular cytoplasm in the form of strands within the tubular lumen. **Abdelhalim and Jarrar (2011)** reported similar findings. According to a study by **Prokić *et al.*, (2019)**, the presence of NPs increased the production of ROS. As a result, this causes the separation and shedding of cells. In addition, the renal cortex showed mononuclear cell infiltration, which correlated with the findings reported by **Ahmed *et al.*, (2022)**. In the current study, the renal medulla showed areas of hyalinization and degeneration in the collecting tubules. The degenerative processes result in necrosis, which can be observed with organelle enlargement, specifically in the mitochondria and endoplasmic reticulum. In addition, lysosomes can rupture prior to the subsequent shrinkage and dissolution of renal cell nuclei. Interstitial haemorrhage was detected in the renal medulla, consistent with the findings reported by **Abdelhalim and Jarrar (2011)**.

However, administration of betaine before PET-NPs leads to a notable reversal of the histological changes caused by PET-NPs in the kidney tissues. As shown by the evidence, the glomeruli are back to their normal size, the architecture of the renal tubules is partly back to normal, and there is less congestion, hyalinization, and the infiltration of inflammatory cells. The results demonstrate the effectiveness of betaine in protecting the kidneys against the toxic effects of PET-NPs. The findings presented in this study align with the results reported by **Hagar *et al.*, (2015) and Ghartavol *et al.*, (2019)**.

Betaine, a substance found naturally in a variety of dietary sources, organisms, and botanical specimens, possesses strong antioxidant properties. In addition, various studies have shown that betaine has impressive lipotropic properties. These properties help protect essential organs like the heart, liver, and kidneys by acting as antioxidants **(Zhao *et al.*, 2018)** through their antioxidant activities **(Abdel-Daim and Abdellatief, 2018)**. In favour of this proposal, previous research has presented findings that demonstrate betaine's ability to alleviate oxidative stress resulting from different factors **(Ozturk *et al.*, 2003; Ganesan *et al.*, 2010)**. The ability of betaine to offer protection against oxidative stress is due to its lipotropic properties. When betaine is provided from an external source, it can readily pass through the cell membrane's lipid bilayer and distribute itself within the cell's internal compartments **(Kanbak *et al.*, 2001)**. One idea suggests that betaine's lipotropic

effects can be attributed to the presence of an electrophilic methyl group, which may help alleviate pathological conditions caused by oxidative stress **(Ghyczy and Boros, 2001)**. Betaine plays a crucial role in the biosynthesis of methionine, which serves as a key supplier of cellular cysteine via the trans-sulfuration pathway. This cysteine is used in the production of reduced glutathione, a compound that protects the cell from reactive metabolites **(Kim and Kim, 2005)**. Our research outcomes support the effectiveness of betaine in improving the increase in MDA levels in renal tissues and the decrease in GSH caused by PET-NPs.

CONCLUSION

In conclusion, giving PET-NPs to Swiss albino mice causes nephrotoxicity, which is shown by increasing levels of BUN, creatinine, and MDA and decreasing levels of GSH. In addition, significant histopathological alterations were identified. Remarkably, the administration of betaine before PET-NPs has shown advantageous effects in alleviating nephrotoxicity generated by PET-NPs. Nevertheless, the outcomes of this study have the potential to facilitate further investigations into the toxicity of various dosages of PET-NPs and the evaluation of alternative protective agents against PET-NPs toxicity.

Consent to participate

Not applicable

Consent for publication

All authors read and approved the manuscript.

Availability of data and materials

Not applicable.

Conflicts of interest

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