Original Article

Effects Of Moringa Oleifera Leaves Extract on Histological Changes Induced in The Distal Convoluted Tubules of The Kidneys Of Adult Albino Rats by Bisphenol

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Abstract

Objective: To evaluate the protective effects of Moringa oleifera leaves extract (MOLE) against BPA-induced histological changes in the DCTs of adult albino rat kidneys.

Methodology: This experimental study was conducted at animal house Postgraduate Medical Institute (PGMI), Lahore from March, 2019 to April, 2020. This study involved 30 male adult albino rats divided into three groups: control group, BPA-exposed, and MOLE-treated plus BPA-exposed. The BPA group received 50 mg/kg BPA orally, while the MOLE-treated group received 400 mg/kg MOLE alongside BPA. Kidney tissues were examined histologically using hematoxylin and eosin staining.

Results: BPA exposure led to notable histological alterations in the DCTs, such as tubular dilation, epithelial cell degeneration, and inflammatory infiltration. In contrast, co-administration of MOLE significantly reduced these changes. The protective effect of MOLE is likely due to its antioxidant components, such as polyphenols and flavonoids, which mitigate oxidative stress and inflammation.

Conclusion: Moringa oleifera leaves extract showed protective potential against BPA-induced renal damage in rats, highlighting its possible therapeutic role in preventing kidney damage caused by environmental toxins.

Keywords: Bisphenol A compound, Distal convoluted tubules, Kidneys, Nephrotoxicity

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Introduction

Bisphenol A (BPA) is a synthetic compound extensively used in the production of plastics, resins, and various consumer goods. Due to its increase usage and ubiquitous occurrence in the environment, its adverse health effects are increasing day by day.[1] Among the affecting organs, kidneys are the most common at risk due to their role in filtering toxins from the bloodstream. Recent studies have BPA in renal dysfunction by fostering the inflammation, cellular toxicity and oxidative stress that commonly affecting the renal tubules.[2] Along with all these, BPA is also involved in the gross changes in renal tissues, particularly on the distal segments of the nephron.[3]

The critical part of the nephron is distal convoluted tubule (DCT) that plays an important role in filtration of electrolytes and reabsorption of fluid, primarily the sodium, chloride, and calcium ions and plays its significant role in maintenance of blood pressure and electrolyte homeostasis.[4] Due to the active transport mechanisms, DCT is particularly susceptible to nephrotoxins.[5] These nephrotoxic injuries are visible on histology, including the dilation of tubules, presence of inflammatory cell infiltrates and degeneration and sloughing of epithelium. All will lead to the compromised renal functions.[6] Moringa Oleifera, also known as the Drumstick tree, is well known for its great pharmacological properties, including anti-inflammatory, antioxidant, and nephroprotective effects.[7] The leaves contain large quantities of biologically potent compounds such as flavonoids, polyphenols, Vitamins (A, C, E), and essential minerals, which aid to neutralize oxidative stress and reduce inflammation[8] Recent studies on animals have shown that Moringa oleifera leaves extract (MOLE) can protect against various nephrotoxins by maintaining the renal functions and architecture.[9] Coming back to BPA-induced renal injury, MOLE may prevent the oxidative damage and maintain the histology of structures like the DCT, so indirectly maintaining renal homeostasis.[10] Because of increased usage of BPA and its deteriorating effects on renal histology especially in the DCT there is a pressing need to investigate the safe and effective interventions. This study aims to assess the protective effects of Moringa oleifera leaves extract against BPA-induced histopathological changes s in the DCT of adult albino rats. Due to the nephroprotective properties of MOLE, the study seeks to decrease the existing gaps in knowledge and help in development of organic, plant-based schemes for the protection of health of kidneys to all those who are exposed to environmental toxins.

Methodology

This experimental study was conducted at the Postgraduate Medical Institute (PGMI), Lahore, after obtaining ethical approval from the Ethical Committee of PGMI and the Advanced Studies and Research Board (ASRB) of the University of Health Sciences, Lahore. This study was conducted from March 2019 to April 2020. A total of 30 adult albino rats of either gender, weighing between 160–180 grams, were procured from the National Institute of Health, Islamabad. The animals were initially examined to rule out any physical abnormalities and were housed in the animal facility of the institute (name to be inserted), where they were kept under standard laboratory conditions. These included a controlled temperature of 28.0 ± 2.0°C, humidity of 60 ± 10%, and a 12-hour light/dark cycle. Rats had free access to a standard pellet diet and water ad libitum. The study duration was 10 months, including one week of acclimatization before the experimental procedures began.

The rats were divided into three groups of ten animals each (n=10). Group A served as the control and received only standard rat feed along with 1 ml/kg of distilled water via oral gavage. Group B, the experimental group 1, received Bisphenol A (BPA) at a dose of 50 mg/kg/day orally.[11] BPA used in this study was in crystalline form and sourced from DAEJUNG, Korea. It was weighed using an ADNF electronic balance, crushed using a mortar and pestle, and suspended in distilled water for administration. Group C, the experimental group 2, received the same dose of BPA (50 mg/kg/day) followed by Moringa oleifera leaves extract (MOLE) at a dose of 500 mg/kg/day.[11,12] Fresh Moringa oleifera leaves were collected daily from the botanical garden of the University of the Punjab, Lahore, and the extract was prepared fresh each day. Both BPA and MOLE were administered via oral gavage for a period of eight weeks.

At the end of the experimental period, all rats were sacrificed. The kidneys were harvested, weighed using an electronic precision balance (Sartorius, Germany), and fixed for histological evaluation. Standard tissue processing and hematoxylin and eosin (H&E) staining protocols were followed. The distal convoluted tubules (DCTs) were analyzed under a light microscope at magnifications of 20X and 40X. For morphometric analysis, micrometry was performed using a calibrated eyepiece and stage micrometer. For each kidney slide, five randomly selected fields were examined, and three clearly defined distal tubules were measured in each field. Tubules with intact and distinguishable boundaries were included. The diameters of DCTs were recorded, and the mean value was calculated for each animal.

Data analysis was performed using SPSS version 25. Quantitative variables, including body weight, kidney weight, and mean diameters of distal convoluted tubules, were expressed as mean ± standard deviation (SD). The normality of data was assessed using the Shapiro-Wilk test. One-way ANOVA was applied to compare the means among the three groups, followed by post hoc Tukey's test for multiple comparisons. p-value of less than 0.05 was considered statistically significant.

Results

Diameter of DCT:

The mean diameter of DCT in all groups was taken. One way ANOVA test was applied to compare the diameter of DCT. There was a marked difference in the mean diameter of DCT in all groups with a p-value <0.001. (Table 1)

DCT Cell Vacuolization

Fisher's exact test demonstrates that an association is present between cell vacuolization of DCT and groups. In group A, cell vacuolization was absent. In all rats of group B, cell vacuolization was present while in group C, only in 2 (20.0%) rats cell vacuolization was present. (Table. 2; Fig. 2)

DCT Nuclear Pyknosis

Fisher's exact test illustrates an association between nuclear pyknosis of DCT among all groups. Group A rats showed no Nuclear Pyknosis in DCT. In group B, Nuclear Pyknosis was present in all rats while in group C 4 (40.0%) rats showed Nuclear Pyknosis. (Table 3; Fig. 3).

Discussion

The heavy metals, chemicals and environmental pollutants/ toxins are now a days the main public health concern globally. In production of polycarbonate plastics and epoxy resins the most commonly used compound is, Bisphenol A (BPA). Other than this it is also frequently used in formation of food containers, water bottles, and baby products.[13,14] Its common adverse effects are disruption of the endocrine system and induction of oxidative stress. Along with these, BPA has been related to various systemic toxicities, including hepatotoxicity, nephrotoxicity, neurotoxicity, and reproductive dysfunction.[15,16]

Kidneys commonly affect organs to xenobiotic-induced injury because they play an important role in the filtration of blood, concentration of urine, and metabolizing substances.[17] In our study, the histopathological alterations noticed in the distal convoluted tubules (DCT) of rats who are exposed to BPA—including tubular dilation, epithelial degeneration, nuclear pyknosis, and cellular vacuolization. These are in accordance with the nephrotoxic effects reported in recent literature. Such structural changes indicate impaired renal tubular architecture and disturbed renal function. These observations are similar to the findings of Saleh et al. (2019) and Ahmed et al. (2020), who stated that BPA-induced degeneration of renal tubular epithelial cells results in disrupted renal histoarchitecture and function.[18,19]

There are so many mechanisms and reasons that will lead to nephrotoxicity caused by BPA. BPA generates oxidative stress by producing reactive oxygen species (ROS), which damage cellular macromolecules such as lipids, proteins, and DNA.[20] Other than this, BPA increases the inflammation through upregulation of pro-inflammatory cytokines like TNF-α and IL-6, and all will increase tissue injury.[21] Oxidative stress and inflammation play main role in apoptosis and necrosis of tubular cells, which ultimately decrease the process of renal filtration and reabsorption.[22]

However, our study concluded that co-administration of Moringa oleifera leaf extract (MOLE) markedly improved these histological damages. The DCTs in rats administrated both BPA and MOLE showed preserved morphology with reduced inflammatory cell infiltration, and decreased cellular degeneration. This nephroprotective effect is in accordance with the past experimental studies emphasizing that MO's is an effective antioxidant, anti-inflammatory, and anti-apoptotic agent.[23,24] Moringa oleifera has powerful bioactive

Table 1. Comparison of diameter of DCT among all groups

Parameters	Group A	Group B	Group C	p-value
Diameter of DCT	30.12 ± 3.44	41.06 ± 3.97	31.55 ± 2.03	< 0.001*

Table 2. Cell vacuolization distribution among all groups

Cell Vacuolization	Group A n (%)	Group B n (%)	Group C n (%)	p-value
Present	0 (0.0%)	10 (100.0%)	2 (20.0%)	<0.001*
Absent	10 (100.0%)	0 (0.0%)	8 (80.0%)	

Table 3. Nuclear Pyknosis distribution among groups

Nuclear Pyknosis	Group A n (%)	Group B n (%)	Group C n (%)	p-value
Present	0 (0.0%)	10 (100.0%)	4 (40.0%)	<0.001*
Absent	10 (100.0%)	0 (0.0%)	6 (60.0%)	

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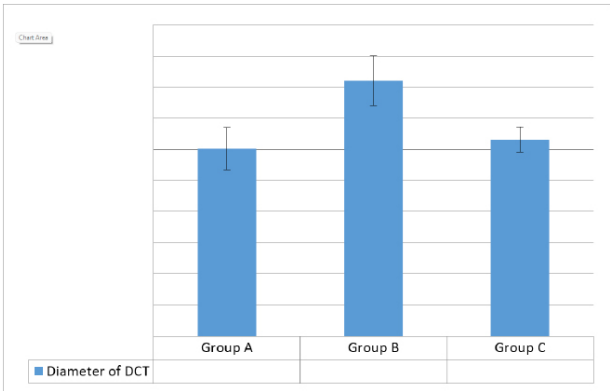


Figure 1: Bar chart showing comparison of diameter of DCT among all group

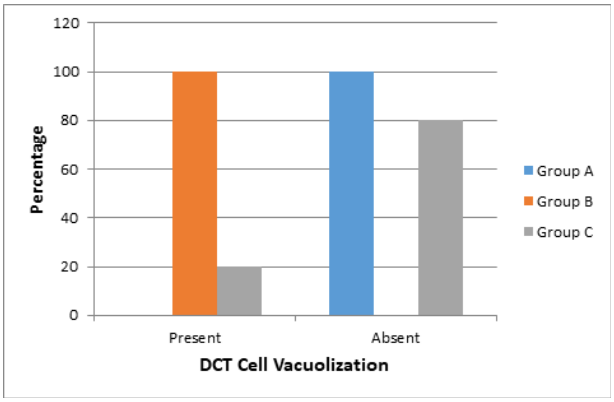


Figure 2: Bar chart showing distribution of DCT cell vacuolization among all groups

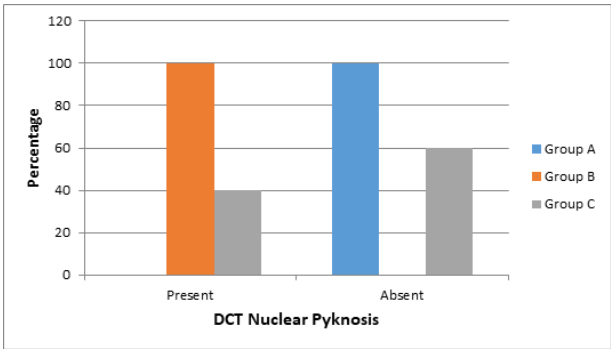
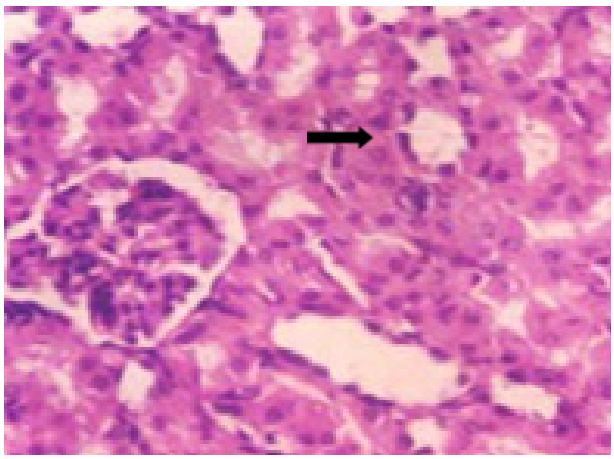
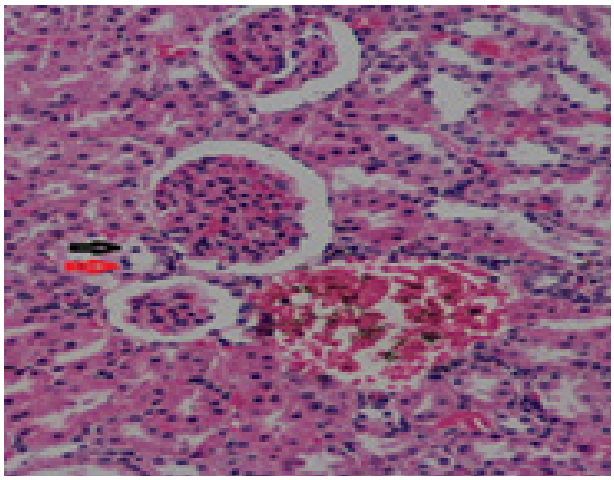


Figure 3: Bar chart showing distribution of DCT Nuclear Pyknosis among all groups

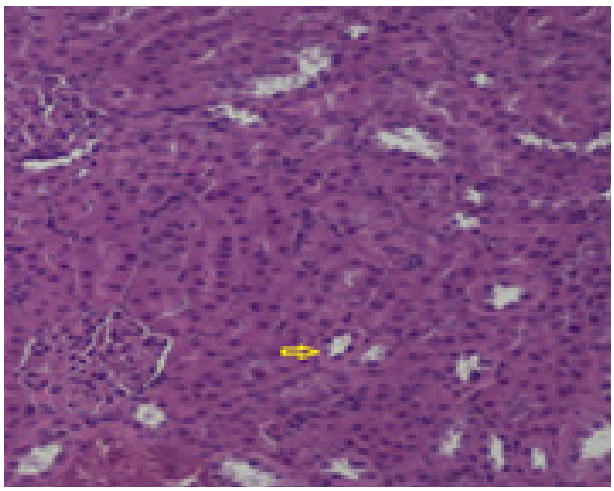
compounds such as flavonoids (quercetin, kaempferol), phenolic acids, vitamins A, C, and E, and minerals like zinc and selenium, all of which have antioxidative properties.[25,26] These compounds produce free radicals, increase endogenous antioxidant enzyme activities (e.g., superoxide dismutase, catalase, glutathione peroxidase), and maintain the integrity of cell membranes, thus preserving renal cells from oxidative damage.[27] The anti-inflammatory abilities of MOLE, led by blockage of NF-κB signaling and downregulation of



A Fig: Photomicrograph of the kidney from the group (A) showing normal DCT (black arrow). H&E stain 40 X.



B Fig. No: Photomicrograph of the kidney from the group (B) showing DCT cell Nuclear Pyknosis (red arrow) and DCT cell vacuolization (black arrow). H&E stain 20X.



C Fig: Photomicrograph of the kidney from the group (C) showing normal DCT (yellow arrow). H&E stain 20X.

pro-inflammatory mediators, all will play their role in renal tissue protection.[28]

Our results are similar to the study of Edeogu et al. (2020) and Owumi et al. (2022), who demonstrated that the administration of MOLE reversed nephrotoxicity induced by chemicals and all this was done by decreasing the activity of oxidative stress markers and inflammatory cytokines.[29,30] Moreover, comparative studies with other antioxidants such as cinnamon and curcumin have showed the same protective effects against renal injury induced by BPA.[31] It's interesting and important that the administration of MOLE significantly decreased the enlarged diameters of renal tubules induced by BPA, which likely shows its role in preventing tubular dilation and edema. This structural protection is very important for maintaining tubular function and overall renal homeostasis. Our findings suggest that the antioxidant defense system of MOLE interferes with the cascade of oxidative damage and inflammation produced by exposure to BPA, thereby preserving both the morphological and functional integrity of renal tissues. Because of usage of MOLE in pharmacology, it is easily available, safe and cost-effective and can easily be used as a making dietary supplement to the high risk patients or those exposed to environmental toxins.[32]

Limitations

While this study provides encouraging evidence, several limitations must be acknowledged. First, only histological changes were evaluated; renal function tests and oxidative stress biomarkers were not assessed. Second, the dose-response effect of MOLE was not investigated. Third, the study duration was short, and long-term consequences remain unknown. Future studies should include biochemical and molecular analyses, explore various doses and treatment durations, compare MOLE with other nephroprotective agents, and eventually extend the research to clinical trials for human applications.

Conclusion

Our findings indicate that Moringa oleifera leaf extract exhibits protective effects against BPA-induced histopathological alterations in the distal convoluted tubules of rat kidneys. The antioxidant and anti-inflammatory properties of MOLE appear to mitigate cellular damage, suggesting its potential use as a natural nephroprotective agent. This study reinforces the importance of exploring dietary or plant-derived therapeutics in combating the harmful effects of environmental pollutants.

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Authors' Contribution Statement

RE contributed to the conception, design, acquisition, analysis, interpretation of data, drafting of the manuscript, critical review, and final approval of the version to be published. MU contributed to the design, acquisition, analysis, and interpretation of data. AMJ contributed to the acquisition, analysis, and interpretation of data. AS contributed to the acquisition, analysis, and interpretation of data. AM contributed to the acquisition, analysis, interpretation of data, and drafting of the manuscript. HAB contributed to the acquisition, analysis, and interpretation of data. All authors are accountable for their work and ensure the accuracy and integrity of the study.

Conflict of Interest

Authors declared no conflict on interest

Grant Suppport and Financial Disclosure

None

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.