Original Article

Protective Effects of Moringa Oleifera Leaves Extract on Bisphenol A Induced Changes in the Proximal Convoluted Tubules of The Kidneys of Adult Albino Rats

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Objective: To determine the effects of Moringa oleifera leaves extract on Bisphenol A induced changes in the PCT diameters and PCT cell vacuolization in the kidneys of adult albino rats.

Methods: Thirty adult albino Wistar rats of any gender, weighing (160-180g) were acquired from National Institute of Health, Islamabad. 30 rats were randomly divided into 3 equal groups as A, B and C, so that each group had 10 rats. Group A rats received standard rat feed and 1ml/kg of distilled water via oral gavage only. Group B received BPA at dose of 50mg/kg/day. Group C received BPA at dose of 50mg/kg/day, followed by MoLE at dose of 500mg/kg/day. All animals were sacrificed at the end of 8 weeks. The kidneys were dissected out and processed further for H&E staining and oculo micrometry was done to measure the PCT diameter and observe the PCT cell vacuolizations.

Results: The increase in diameters of PCT and PCT cell vacuolization, that was seen in group B upon giving BPA only was prevented in the animals of group C that was given MoLE with BPA.

Conclusions: Moringa oleifera leaves extract prevents BPA induced increase in the PCT diameters and PCT cells vacuolizations in the kidneys of adult albino rats.

Keywords: bisphenol a, moringa oleifera leaves extract, albino rats, proximal convoluted tubules.

Introduction

Human beings are constantly being exposed to Bisphenol A [BPA, 2, 2-bis (hydroxyphenyl) propane.[1] It contains phenolic rings and is an environmental toxin.[2] This compound is used to prepare epoxy resins (e.g., inner linings of metallic cans) and polycarbonate plastics and also in the form of non-polymer additive to various plastics. It is also present in drinking water. It is a constituent of infants and water bottles, kitchen ware, food containers and dental materials.[3] BPA causes oxidative stress and has injurious effects on kidneys, liver, and reproductive system.[4]

According to the studies on rodents, NOAEL for BPA is stated to be 5mg/kg and a lethal dose (LD50) is considered as 3.25 g/kg in rats.[5] In both, rats and humans, BPA is absorbed from gastrointestinal tract and conjugated in liver enzymatically to a glucuronide form which is excreted in urine.[6] Moringa oleifera is a valueable tropical tree which is being used as human medicine, food and also in oil production. It is known to be 'Sohanjana' in Pakistan and is grown widely all over the country. Moringa oleifera is commonly and widely found in India, Pakistan, Asia, Africa and Arabia.[7] Leaves of this tree are antihyperglycemic, hypocholesterolemic, antitumor agent, antioxidant.[8]

Methods

Experimental study was conducted at the animal house and histology lab of PGMI, Lahore. 30 adult albino rats of either sex, weighing 160 180gms were obtained from the National Institute of Health (NIH), Islamabad. Rats were provided with food and water ad libitum. Male and female rats were kept separately in iron cages under optimum temperature (24±2°C) and 12 hours light/dark cycle. Following acclimatization for a period of one week experiment was started. Each rat was weighed at start and end of experiment. Rats were divided into 3 groups comprising of 10 rats in each group **(Table-1).**

Table-1: Showing detail of the animal groups and experimental Intervention.

Group n=10	Administration	Week of scrifice	Intervention and dosage
Control B		End of 8 weeks	2ml/kg distilled water only ad libitum
Experimental C	Orally		BPA 50 mg/kg suppended in distilled water
Experimental			BPA 50 mg/kg+MOLE 500mg/kg dissolved in distilled water

Therapeutic Agents

BPA: Daejung Company, Korea.

Preparation of Moringa Oleifera Leaves Extract:[9]

Fresh Mo Leaves were procured from botanical gardens of the University of Punjab. Leaves were washed, shadow dried for 2 weeks to prevent direct

sunlight, to prevent them from damage. Leaves were ground to powder and carried to PCSIR lab complex Lahore for further processing.

Tissue Sampling: The kidneys of sacrificed rats were dissected out for detailed morphological and histological observation. For histological examination, the tissues was fixed in neutral buffer 10% formaldehyde solution and processed to make paraffin embedded blocks. Slides were stained with standard procedures of Hematoxylin and Eosin.

Mirometery of kidneys was done to determine diameters of proximal convoluted tubules.

For each slide, proximal tubules were observed in five randomly selected different fields. From each field, the diameters of three proximal tubules were determined at magnification of 40X. Tubules with clear boundaries were selected. At the end, mean size was calculated.

Results:

Diameter of PCT: One way ANOVA test was applied to compare the diameter of PCT among groups. It was found that the mean diameter of PCT in all groups were significantly different (p-value < 0.001) **(Table-2, Fig-1).** For multiple comparisons, post hoc Tukey test was used which showed that diameter of PCT in group B was significantly higher as compared to group A and C. However, no significant difference was found in the diameter of PCT among groups A and C **(Table - 3).**

PCT Cell Vacuolization: Fisher's exact test showed that there was an association between cell vacuolization of PCT and groups **(Table-4, Fig-2).** Cell vacuolization of PCT in rats of control group A was absent (Fig. 3 A). In group B, cell vacuolization was present in all rats. While in group C, only in 2 (20.0%) rats cell vacuolization was present in PCT **(Fig-3B and 3C).**

Table-2: Comparison of diameter of PCT among groups using one way ANOVA.

Parameters	Group-A	Group-B	Group-C	P-value
Diamter PCT(µm)	34.75±3.97	45.51±5.08	37.30±3.26	<0.001*

Table-3: Pair wise comparison of diameter of PCT among groups

Multiple Comparison	Groups-I	Groups-J	Mean difference (I-J)	Std. Error	P-value
Diameter of PCT	A	B	-10.7600	1.8652	0.000*
		C	-2.5500	1.8652	0.372
	B	C	8.2100	1.8652	0.000

**p value ≤ 0.05 is considered statistically significant*

Table-4: Distribution of PCT cell vacuolization among groups: (Fisher's exact test).

Cell Vacuolization	Group-A	Group-B	Group-C	P-value
Present	0 (0.0%)	10 (1001%)	2 (20.0%)	<0.001*
Absent	10 (100.0%)	0 (0.0%)	8 (80.0%)	

**p value ≤ 0.05 is considered statistically significant*

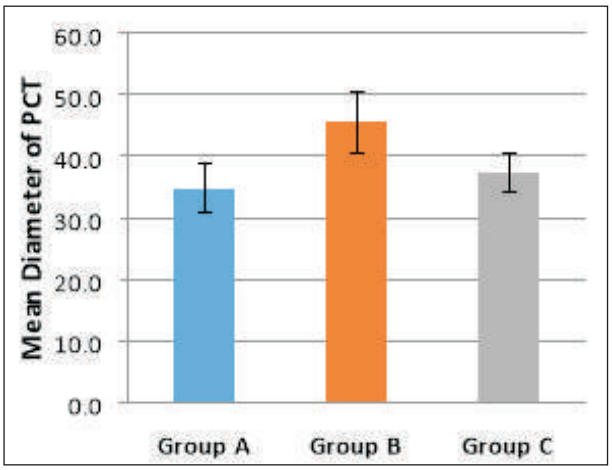

Fig-1: Bar chart showing comparison of diameter of PCT among groups.

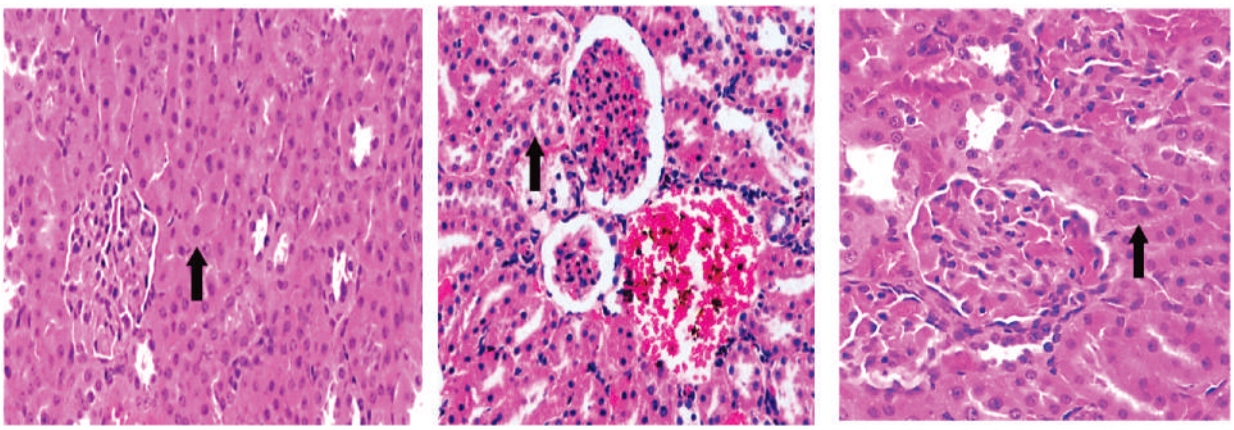

Figure 2: bar chart showing distribution of pct cell vacuolization among groups.

Discussion

The diameters of proximal convoluted tubules were measured and compared among groups in this study. The statistical analysis showed that these PCT diameters of control (34.75±3.97µm) and experimental groups receiving *Mo* along with BPA (37.30±3.26µm) were nearly similar while that in experimental group receiving only BPA, were markedly increased (45.51±5.08µm). Similarly, the results of experimentation done by Helal et al[10] and Hassan et al[11] also showed an increase in PCT diameter upon giving BPA to experimental rats. This increase in tubular diameter might be explained because of increase in tubular cell sizes due to BPA and later on degeneration of tubular cells. This might have occurred due to accumulation of BPA metabolites and inability of the rat kidneys to eliminate them causing necrosis and degeneration of renal tubules.[9,11] Similarly, dilated renal tubules were observed by Ahmed et al.[7]

On the other hand, a study conducted by Anibese et al[12] on nephro protective effects of *Mo* on potassium bromate induced kidney damage illustrated normal kidney architecture of animals that were given *Mo* which supports the present study. The proposed mechanism of *Mo* to prevent nephrotoxicity might be due to its antioxidant properties.[13] The seeds of *Moringa* contains many antioxidants like tocopherols, vitamins C, E and polyphenols possessing radical trapping ability.[14] Presence or absence of vacuolization of proximal tubular epithelial cells was noted in this study, in the kidneys of each rat in both experimental groups B (BPA alone) and C (BPA+*Mo*). It was found that all of the rats in group B had proximal tubular cellular vacuolization indicating the active inflammatory process. While, only two of the experimental rats in group C had vacuolization present in their kidneys. Whereas, proximal tubular cell vacuolization was not found in any of the rat in control group A. Vacuolization in renal tubules after BPA administration was also present in the study done by Helal et al.[10] Korkmaz et al[15] and Ola-Davies et al.[16] Cause behind this vacuolization as a marker of kidney injury probably is the oxidative stress induced by ROS produced in mitochondria and microsomes of renal tubular cells which damage the nucleic acids, proteins and lipids.[16]

Conclusion

Moringa oleifera leaves extract prevents BPA induced increase in the PCT diameters and PCT cells vacuolizations in the kidneys of adult albino rats.

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