ORIGINAL ARTICLE

Proximal Convoluted Tubules as the Primary Target of Renal Histopathological Injury by Ingestion of Pesticide-Residue-Laden Fruits and Vegetables

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ABSTRACT

Objective: To determine the potential adverse renal histopathological effects on the proximal convoluted tubules in male, *Wistar* rats due to ingestion of subchronic dose of triazophos.

Study Design: Randomized controlled trial.

Place and Duration of Study: A study of 3 weeks duration (plus 1 week for slide preparation & histological study), was held in the animal house-PGMI, Bird wood road, Lahore.

Materials and Methods: Two equal groups were made, of 6 *Wistar*, male rats each. Group A was control while experimental group B was given triazophos, in a dose of 8.2 mg/kg-subchronic dose ($1/10^{th}$ of LD 50), a commonly used pesticide, as 1:100 dilution solution through oral gavage for 21 consecutive days. On day 22, the rats were sacrificed and tissues preserved for histological examination and measurement of proximal and distal tubular diameters by using micrometric grid.

Results: The micrometric measurement of diameters of PCT in group A and B indicated that they increased significantly from 38.90 ± 2.72µm in group A to 50.35 ± 2.61µm in group B (p-value <0.001). The mean diameter of DCT in both the groups was also measured and increased in group B from 34.48 ± 2.58µm in group A to 48.03 ± 3.42µm in group B (p-value <0.001). In group B, inflammatory changes like basement membrane interruption, loss of brush border and severe vacuolar cellular degeneration of proximal convoluted tubules were seen.

Conclusion: The results confirm the potential adverse renal histopathological effects on the proximal convoluted tubules in male, *Wistar* rats due to ingestion of triazophos, a commonly used pesticide. Pesticides induce inflammatory changes in convoluted tubules leading to tubular dilatation and hyperplasia more in PCT owing to the extensive exposure of toxic substances in the proximal tubules and resultant increased PCT mean diameter.

Key Words: *Food Chain, Harmful Residues, Insecticides, Pesticides, Renal Damage, Triazophos.*

Introduction

The scientists claim that organophosphorus pesticides like triazophos rapidly degrades and do not tend to persist or bioaccumulate in the environment or food chain but unfortunately pesticide residues in food are regularly detected at low levels in the food chain in a range of food items and water.[1] Triazophos is an organophosphorus pesticide, sprayed rampantly on crops to get rid of the pests infesting on them and to improve the crop yield.[2] It is widely used in agriculture, veterinary medicine and public health all over the world due to its rapid biodegradability and good control over the pests.[3] In spite of all the claims of pesticides' non-persistence in the food chain, their residues' presence in food items cannot be denied. Thus, not only the nutritional value of the food is compromised, pesticides' presence adversely affects the environment as well as the consumers' health.[4] Liver & kidneys are the primary organs to be affected due to the conjugative metabolism & excretion of the pesticides through these organs.[5] In kidneys, proximal convoluted tubules are the primary target of histological injury owing to their greater length than the distal convoluted tubules & greater

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exposure of all kinds of toxins to them.[6]

The unfortunate extension of pesticides' harmful effects, from the target of pest control to involvement of non-target spectrum of other living organisms as well has led to a confusion whether pesticides are more harmful or useful for the mankind.7 The rationale of present study aimed at observing renal damage especially in the proximal convoluted tubules when the experimental animals ingested subchronic dose of triazophos for a period of 3 weeks. The main objective of the study was to determine the potential adverse histopathological effects on the proximal tubules in male, *Wistar* rats due to ingestion of subchronic dose of triazophos.

Materials and Methods

The study was randomized controlled trial of 3 weeks duration (plus 1 week for slide preparation & histological study) held in the animal house of PGMI, Lahore, in which 12 male, *Wistar* rats of age 6-8 weeks (excluding the females, < 6 weeks old, < 150 g weight, sick and ailing rats), weighing 175-200 g were selected. Rules and regulations laid down by the Ethical review board of Postgraduate medical institute, Lahore were followed & their ethical permission taken. The sampling technique adopted was by simple random sampling, with the help of Stat Trek's Random Number Generator. Group A was kept as control and group B as experimental.

The animals were acclimatized for a week; food and water were made available *ad libitum,* with maintenance of 12-hour light and dark cycle and temperature maintained at 25-30°C. Triazophos was given in the sub chronic dose, 8.2 mg/kg body weight ($1/10^{th}$ of LD50)[8] to the group B as dilution solution through oral gavage while same quantity of distilled water was given to the group A for 21 consecutive days.[9] Parametric data was noted on weekly basis. The rats were sacrificed on day 22, their kidneys dissected out and preserved for H & E slides preparation and histological examination for micrometric measurement of tubular diameters.

Four proximal and four distal convoluted tubules (sub capsular, mid-cortical, juxtamedullary and medullary) [10] were selected per high power field (40X); for each slide; both the vertical and horizontal diameter between the opposite basement membranes of each tubule was measured and then average taken. Finally, the mean diameters of PCT and DCT were determined for each animal.[11] For micrometry**,** an eyepiece micrometer scale and a stage micrometer slide were used. On the stage micrometer slide, a 1 mm scale was engraved. This 0.01 mm scale was divided into 100 equal divisions. 15 divisions of eyepiece micrometer were equal to 4 stage micrometer divisions.[12]

100 stage divisions = 0.01mm = 1000 µm

1 stage division = 1000/100 = 10 µm

15 divisions of eyepiece micrometre = 4 stage divisions (40 µm)

1 division of eyepiece micrometre = 40/15 = 2.66 µm

The measured average diameter of each tubule was multiplied by 2.66 to get the exact tubular diameter.[13]

Data was analyzed by SPSS version 22.0. Independent sample t-test was used to compare the mean diameters of PCT and DCT in both groups. Fisher's exact test was used to analyze the proximal and distal tubular epithelial cells for cytoplasmic vacuolization and loss of brush border.

Results

Diameter of PCT/DCT: The mean diameters of PCT and DCT in both group A and B were taken.

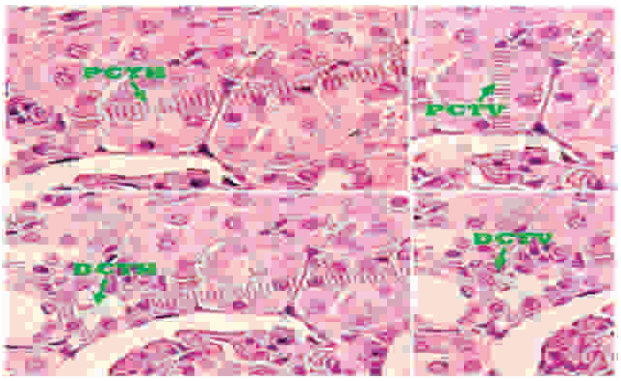

Fig 1: Photomicrograph Showing the Horizontal and Vertical Proximal Convoluted Tubular Diameter (PCTH/ PCTV) and Distal Convoluted Tubular Diameter (DCTH/ DCTV) through Micrometer. H&E X400

Table I: Mean Diameter Comparison PCT and DCT among Groups

Parameters	Group A	Group B	p-value
Mean diameter PCT of all rats in a group	38.90 ± 2.72	50.35 ± 2.61	< 0.001*
Mean diameter DCT of all rats in a group	34.48 ± 2.58	44.03 ± 3.42	< 0.001*

Independent sample t test

*p *value* ≤ 0.05 is considered statistically significant

Loss of Brush border

Fisher's exact test showed that there was statistically significant association between loss of brush border PCT and groups (Table II Figure 2, 3)

Table II: Distribution of Loss of Brush Border (PCT) Among Groups

Brush Border loss	Group A N=6 (%)	Group B N=6 (%)	*p-value*
Normal	6 (100%)	0 (0.0%)	0.002*
Mild 25-50%	0 (0.00%)	0 (0.0%)	
Moderate 50-75%	0 (0.00%)	2 (33.3%)	
Severe 75-100%	0 (0.00%)	4 (66.7%)	

Fisher's exact test

*p value ≤ 0.05 is considered statistically significant

Cytoplasmic Vacuolization (PCT/DCT):

Fisher's exact test showed that there was statistically significant association between cytoplasmic vacuolization in PCT and DCT among groups. (Table III, IV; Figure 2, 3)

Table III: Distribution of Cytoplasmic Vacuolization (PCT) Among Groups

Cytoplasmic vacuoles in PCT	Group A N=6 (%)	Group B N=6 (%)	*p-value*
Normal	6 (100%)	0 (0.0%)	0.002*
Mild 25-50%	0 (0.00%)	0 (0.0%)	
Moderate 50-75%	0 (0.00%)	1 (16.7%)	
Severe 75-100%	0 (0.00%)	5 (83.3%)	

Fisher's exact test

*p value ≤ 0.05 is considered statistically significant

Table IV: Distribution of Cytoplasmic Vacuolization (DCT) Among Groups

Cytoplasmic vacuoles in DCT	Group A N=6 (%)	Group B N=6 (%)	*p-value*
Normal	6 (100%)	0 (0.00%)	0.002*
Mild 25-50%	0 (0.00%)	0 (0.00%)	
Moderate 50-75%	0 (0.00%)	2 (33.3%)	
Severe 75-100%	0 (0.00%)	4 (66.7%)	

Fisher's exact test

*p value ≤ 0.05 is considered statistically significant

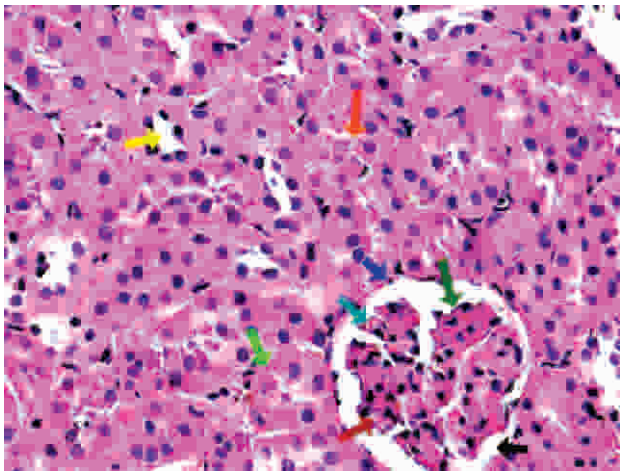

Fig 2: Photomicrograph of group A kidney cortex showing glomerulus **(Red)**, parietal **(Blue)** and visceral **(Dark-Green)** layers of Bowman's capsule **(Black)**, tuft of capillaries **(Aqua-Blue)**, proximal tubules (**Light-Green**) with brush border **(Orange)** and distal convoluted tubules **(Yellow)**. H&E stain X400.

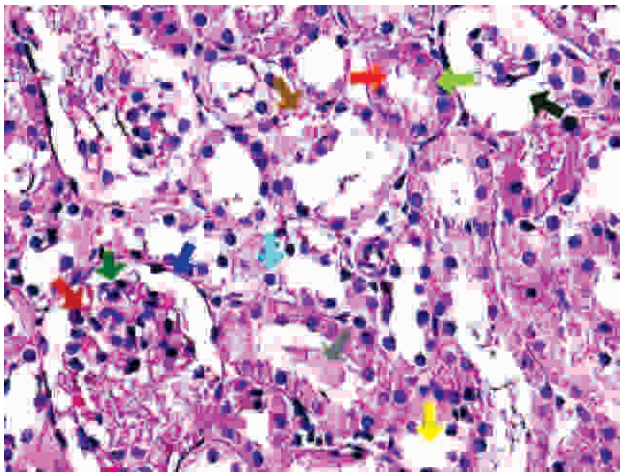

Fig 3: Photomicrograph kidney cortex in group B showing glomerulus **(Red)**, parietal **(blue)** and visceral layers **(Dark-Green)**, Bowman's space **(Black)**, glomerulus **(Pink),** distal **(Yellow)** & proximal tubules (**Light-Green**) loss of brush border (**Orange),** protein cast **(Grey)**, congestion in the blood vessel (**Brown**) and vacuole **(Aqua Blue)**. H&E stain X400.

Discussion

In the present study, subchronic dose of triazophos induced nephrotoxicity evidenced by histopathological changes in the kidneys.[14] The most marked change observed in the H & E kidney slides of rats of group B was increased tubular diameter measured by using micrometric grid under light microscope (Table I, Fig. 1), due to cellular hyperplasia leading to tubular dilation & ultimate

epithelial necrosis, degeneration and desquamation with granular debris in the tubular lumen. This increase in diameters was statistically significant (p-value <0.001).

Pesticides exhibit their harmful effects due to induction of oxidative stress in the cells and generation of free oxygen radicals.[16] The reactive oxidative radicals cause anatomical and functional alterations in the mitochondria of cells.[17]

In group B, all rats had moderate to severe interrupted brush border (Table II, Fig 3).[15]This loss of brush border was statistically significant (p value< 0.002). Cytoplasmic vacuolization of PCT (Table III, Fig 3) and DCT (Table. IV, Fig 3) in the rats of group B was also statistically significant.

The loss of brush border results when lysosomal degradative enzymes damage the glycocalyx thus disrupting the microfilaments making the structure of microvilli onto the cytoskeleton of cells as well as cell membrane disruption.[19] Disturbed ionic balance in the organelles underlie the formation of multiple membrane vesicles which fuse to form vacuoles causing breakdown of organelles and finally cell death as already explained by Elhalwagy in 2016 in a study on effects of triazophos on liver and kidneys of rats.[20]

Commonly used pesticides like triazophos, persist in the environment and food chain.[21] Jain et al, in 2010.[17] Ghaffar et al, in 2014[22]and Mohineesh et al, in 2014[23] conducted studies on triazophos and its subchronic dose's effects on body tissues. They all proved that triazophos adversely effects the body organs. Rahman and Sattar in 2018 conducted a study on effects of different doses of pesticides on body tissues.[24]

The present study was conducted to observe the persistence of organophosphorus pesticides in the food chain of Pakistan, the present status of which is quite alarming for the health of mankind, as rampant usage of pesticides on crops is tenfold high in Pakistan as compared to other countries. The limitations of the present study were the unawareness among the general population about the hazards of the rampantly-used pesticides and insecticides on almost all the crops and the persistence of their residues in the fruits and vegetables. The gravity of the situation and the unawareness towards its seriousness seems like a hidden iceberg and need further probing into the matter.

Conclusion

The results confirm the potential adverse renal histopathological effects on the proximal convoluted tubules in male, *Wistar* rats due to ingestion of triazophos, a commonly used pesticide. Pesticides induce inflammatory changes in convoluted tubules leading to tubular dilatation and hyperplasia more in PCT owing to the extensive exposure of toxic substances in the proximal tubules and resultant increased PCT mean diameter.

REFERENCES

1. Zia MS, Khan MJ, Qasim M, Rehman A. Pesticide residue in the food chain and human body Inside Pakistan. *J. Chem. Soc. Pak*. 2009; 31:284-91.
2. Syed JH, Alamdar A, Mohammad A, Ahad K, Shabir Z, Ahmed H, et al. Pesticide residues in fruits and vegetables from Pakistan: a review of the occurrence and associated human health risks. *Environ. Sci. Pollut. Res.* 2014; 21(23):13367-93.
3. Kim KH, Kabir E, Jahan SA. Exposure to pesticides and the associated human health effects. *Sci. Total Environ*. 2017; 57:525-35.
4. Khan M, Mahmood HZ, Damalas CA. Pesticide use and risk perceptions among farmers in the cotton belt of Punjab, Pakistan. *Crop Prot.* 2015; 67:184-90.
5. Bock R, Thier W. Metabolism and fate of triazophos in rats. *Pestic. Sci.* 1976; 7(3):307- 14.
6. Chevalier RL. The proximal tubule is the primary target of injury and progression of kidney disease: role of the glomerulo-tubular junction. *Am. J. Physiol. Renal Physiol*. 2016; 311(1):145-61.
7. Damalas CA, Eleftherohorinos IG. Pesticide exposure, safety issues, and risk assessment indicators. *Int. J. Environ. Res. public health.* 2011; 8(5):1402-19.
8. Chandra MO, Raj JA, Dogra TD, Rajvanshi AC, Raina AN. Determination of median lethal dose of triazophos with DMSO in wistar rats. *Asian. J. Pharm. Clin. Res*. 2014; 7(4):64-7.
9. Sharma D, Sangha GK. Triazophos induced oxidative stress and histomorphological changes in liver and kidney of female albino rats. *Pestic. Biochem. Physiol.* 2014; 110:71-80.
10. Rahman MA, Rahman MA, Karim F. Histomorphological Study of the Wall Thickness and Luminal Diameter of Cadaveric Vermiform Appendix in Bangladeshi People.

American Journal of Medical Sciences. 2019;7(3):89-93.

11. Qadir MI, Tahir M, Lone KP, Munir B, Sami W. Protective role of ginseng against gentamicin induced changes in kidney of albino mice. Journal of Ayub Medical College Abbottabad. 2011;23(4):53-7.
12. Bhattacharya T, Bhakta A, Ghosh SK. Long term effect of monosodium glutamate in liver of albino mice after neo-natal exposure. Nepal Med Coll J. 2011; 13(1):11-6.
13. Gulnaz H, Tahir M, Munir B, Sami W. Protective effects of garlic oil on acetaminophen induced nephrotoxicity in male albino rats. *Biomedica*. 2010; 26(7):9-15.
14. Mossa AT, Swelam ES, Mohafrash SM. Subchronic exposure to fipronil induced oxidative stress, biochemical and histopathological changes in the liver and kidney of male albino rats. *Toxicol. Rep.* 2015; 2:775-84.
15. Beceren A, Sehirli AO, Omurtag GZ, Arbak S, Turan P, Sener G. Protective effect of N- Acetyl-L-Cysteine (NAC) on endosulfan-induced liver and kidney toxicity in rats. *Int. J. Clin. Exp. Med.* 2017; 10(7):10031-9.
16. Rashid S. Effect of alpha-tocopherol on diameter of proximal convoluted tubules of kidney in diabetic mice. *J Pak Med Assoc*. 2014; 64:49-52.
17. Jain S, Mythily S, Ahmed RS, Arora VK, Banerjee BD. Induction of oxidative stress and histopathological changes by subchronic doses of triazophos. *Ind. J. Biochem. Biophysic.* 2010; 47:388-392.
18. Rathod GB, Parmar P, Rathod S, Parikh A. Hazards of free radicals in various aspects of health–a review. *J Forensic Toxicol Pharmacol.* 2014; 3(2):1-7.
19. Yasmeen T, Qureshi GS, Perveen S. Adverse effects of diclofenac sodium on renal parenchyma of adult albino rats. *J. Pak. Med. Assoc*. 2007; 62: 42-47.
20. Elhalwagy ME. Subchronic impact of organophosphorus insecticide Triazophos on liver, kidneys and thyroid of albino rats. *Int. J. Adv. Res. Biol. Sci.* 2016; 3(1):199-208.
21. Raj J, Rajvanshi AC, Dogra TD, Raina A. Effect of acute exposure of triazophos on oxidative stress and histopathological alterations in liver, kidney and brain of Wistar rats. *Ind. J. Exp. Biol*. 2014, 814-819.
22. Ghaffar A, Ashraf S, Hussain R, Hussain T, Shafique M, Noreen S, et al. Clinico-hematological disparities induced by triazophos (organophosphate) in Japanese quail. *Pak. Vet. J.* 2014; 34:257-9.
23. Mohineesh, R.J., Rajvanshi, A.C., Dogra, T.D. and Raina, A. Effect of acute exposure of triazophos on oxidative stress and histopathological alterations in liver, kidney and brain of *Wistar* rats. *Indian J. Exp. Biol.*, 2014; *52*(8):814-819.
24. Rahman SA, Sattar DA. Effect of Different Concentration of Super Cyren Pesticide on Some Physiological and Histological Traits of Mice after Different Periods of Oral Administration. *Iraqi J. Sci*. 2018; 58(4C):2291-300.