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Research Article

ASSESSMENT OF RENAL TOXICITY IN RATS EXPOSED TO COMMERCIAL FORMULATIONS OF FIPRONIL

Kartheek RM and David M*

Environmental Toxicology and Molecular Biology Laboratory, Department of PG Studies and Research in Zoology, Karnatak University, Dharwad- 580003, Karnataka, India.

ABSTRACT

Renal dysfunction as a consequence of pesticide exposure has been often discussed in scientific community. Nevertheless, literature support for same on new class insecticides have been in limited proportion. Fipronil (FPN), a new class phenylpyrazole insecticide is known for its action against insect pests that possess resistance ability over the conventional group of pesticides. An attempt was made in the current study to investigate the influence of FPN on biochemical and histopathological faction in kidney of Wistar rats. Experimental animals were divided in four groups; C, E1, E2 and E3 and received 0.0, 6.6, 12.12 and 32.33 mg/kg body weight of FPN respectively through oral gavage for 90 days. Results on antioxidant enzymes suggested significant decline (*p<0.01) in catalase, superoxide dismutase and glutathione peroxidase activity in the kidney of exposed rats under groups E2 and E3 unlike E1 as compared to C. Similarly, a significant (*p<0.01) elevation in malondialdehyde level was also noticed indicating oxidative damage potential of FPN in rats under E2 and E3. The outcome was verified through histopathological investigation which demonstrated anomalies including damaged proximal and distal convoluted tubule, increase in tubular lumen, fibrosis and necrosis. Based on the outcome, it can be inferred that FPN inflicts dose dependent damage on kidney of rats under subchronic exposure duration and hence poses a potential threat. It is therefore recommended that care should be taken whenever FPN is used or disposed under mammalian proximity.

Keywords: Fipronil, Kidney, Malondialdehyde, Nephrotoxicity, Xenobiotics.

1. INTRODUCTION

Indiscriminate pesticide use has resulted in unfavourable environmental conditions causing adverse health effects on non-target organisms (Schwarzenbach et al., 2010; Kartheek and David, 2016). Due to their extensive usage in agriculture and persistent nature, pesticides are known to pose a serious toxicological threat to integrity of environment and more importantly to its biota (David and Kartheek, 2015; Albuquerque et al., 2016). Previously, several studies have been carried out to understand the toxicity of conventional group of pesticides on non-target species (David and Kartheek, 2015). However, the toxicants evaluated have been with the objective of elucidating hepatic (Kammon et al., 2010; David and Kartheek, 2014), neuro (Lee et al., 2016), cardiovascular (Zafiropoulos et al., 2014) and reproductive systems (Sharma et al., 2014). Investigations on toxic potential of new class insecticides in causing renal dysfunction have been rarely reported so far.

Numerous pesticides have been identified to generate free radicals within biological system (Mansour and Mossa, 2009; David and Kartheek, 2016). Several chemically non-related compounds possess the ability to induce oxidative stress which has prompted the use of antioxidant and oxidative damage responses as non-specific, yet sensitive biomarkers, useful to characterize impacted environments with complex mixtures of contaminants (Viegas-Crespo et al., 2003; Ferreira-Cravo et al., 2007). There is a growing interest among researchers regarding the role of oxidative stress and reactive oxygen species (ROS) in the

pathophysiological mechanism (Deng et al., 2010).

Fipronil (5-amino-1-[2, 6-dichloro-4(trifluoromethyl) phenyl]-4-[(trifluoromethyl) sulfinyl]-1H-pyrazole-3-carbonitrile)) (FPN) a phenylpyrazole insecticide, has been widely used in many countries for its ability to counter insect pests that have over time gained resistance to conventional pesticides (Stark and Vargas, 2005). FPN is known to act on the nervous system through a non-competitive binding to the gamma-aminobutyric (GABA) receptor, and thus blocking the normal passage of chloride ions and the transmission of normal neural impulse (Kidd and James, 1991) resulting in paralysis and finally death. Evaluation of FPN toxicity have been conducted by various authors across the globe, elucidating its toxic impact on hepatic and neuro behavioural aspects. Nevertheless, its ability to induce renal damage has not been reported so far to the best of our knowledge.

Hence in the present investigation, an attempt has been made to evaluate the FPN impact on kidney of wistar rats exposed under subchronic tenures by evaluating oxidative stress potential and histopathological potentials.

2. MATERIALS AND METHODS

2.1 Animal procurement

Male Wistar albino rats (8 weeks) were obtained from animal house facility, Department of PG studies and Research in Zoology, Karnatak University, Dharwad, Karnataka, India. Rats were housed in the polypropylene cages with ad libitum access to standard pellet feed and drinking water. The room was maintained under a 12/12 h light– dark cycle, an ambient temperature of 23-30 °C with a relative humidity of 45.0 (±15)%.

2.2 Experimental design and test doses

For a 90-day study of oral toxicity, the 6-7 week old Wistar rats were randomly assigned into four groups (C, E1, E2 and E3) of six males each. While group C of untreated rats served as the control group, rats under group E1, E2 and E3 were exposed to of 0.0, 6.46, 12.12 and 32.33mg/kg body weight of FPN dose in drinking water.

2.3 Antioxidant enzyme assay

Catalase (CAT) activity was determined by measuring the decrease of hydrogen peroxide concentration at 240nm according to Luck (1974). Superoxide dismutase (SOD) activity was measured by methodology as described by Kakkar et al. (1984). Glutathione peroxidase (GPx) activity was measured by the protocol stated by of Paglia and Valentine (1967). Glutathione-S-transferase (GST) activity was assayed by the method of Habig et al. (1974). LPO level was performed according to the method of Buege and Aust (1978).

2.4 Histopathology

For the histopathological examination, the method was followed as described by Humason (1972). Kidney was isolated and immediately fixed in Bouin's fluid for 24 to 48 h. The tissue was processed in a series of graded alcohol and embedded in paraffin which was being filtered thrice earlier. The organs embedded in paraffin were sectioned into 5-µm-thick ribbons by using semi-automated microtome (LeicaRM 2255), and sections were stained primarily with haematoxylin and counter-stained with eosin (H&E) for light microscopic examination (Lille 1969). The sections were observed under ×200 magnification. The microscopic view was photographed by using an Olympus phase contrast microscope (Olympus BX51, Tokyo, Japan) with attached photography machinery (ProgResC3, Jenoptic- Germany). The photographed images were further observed for differences, and the findings were recorded. The photomicrograph were processed with adobe Photoshop 7.0 for slight management in brightness and contrast.

3. RESULTS AND DISCUSSION

3.1 Antioxidant status

Applications of antioxidants are many and their formulations are being used in prevention and treatment of complex diseases including stroke, diabetes, Alzheimer's and cancer (Devasagayam et al., 2004). Mechanism of action of these antioxidants is by neutralizing free radicals that exist in surplus amounts, thereby defending the cells against establishment of oxidative stress (Kartheek and David, 2016). Significant (*$p<0.01$) difference in enzymatic activity of CAT, SOD and GPx was recorded in kidney of rats under group E2 and E3 unlike E1 as compared to C. The changes noticed were found to be in dose dependent pattern. Further, to elucidate, changes in CAT activity noticed were significant (*$p<0.01$) and highly significant (**$p<0.001$) for E2 and E3 with percent change of 26.8 and 57.34 % respectively. However, a slight increase in CAT activity was noticed in E1 rats with percent change of 1.44% was noted and was not found to be significant (*$p<0.01$). CAT being a peroxisomal enzyme is mainly localised in liver and kidney of eukaryotic cells, hence protecting these vital organs against excess of ROS abuse (Schrader and Fahimi, 2006). Changes in magnitudes of CAT has been

correlated with damage under cellular levels often instigating biochemical imbalance resulting in tissue catastrophe (Mittal et al., 2014). The current investigation therefore indicates FPN toxicity on renal tissue through decline in CAT enzymatic threshold thereby suggesting a possibility of peroxisomal damage and has been previously suggested by David and Kartheek (2016).

Similar trend was noticed in SOD activity which showed a decline of -42.11 and -69.72% for E2 and E3 respectively under FPN stress. However, rats under E1 demonstrated a very mild elevation in SOD which was recorded to be 0.48% with no significant (*p<0.01) difference when compared to group C. The changes in terms of SOD activity has been often linked to occurrence and progression of higher superoxide radicals (Djordjević et al., 2017). Previously, long-term exposure study on pesticides have suggested inhibition of SOD activity causing oxidative damage to kidney (Shah et al., 2007) which supports our present study. SOD has proven to be a useful probe for studying the free radicals in reactions involving oxygen, since it acts as a defence against oxidative tissue damage by dismutation of superoxide radicals (Uttara et al., 2009).

SOD and CAT act in tandem with each other and their existence in the system is critical for dismutation of ROS that has been generated additionally under the influence of toxicants (Muradian et al., 2003). The current outcome indicated imbalance in addition to decline in the complex threshold of CAT and SOD which is consistent with the reports of Mahmoud et al., (2015) who observed declining magnitudes of one of the antioxidant enzyme under the influence of diethylnitrosamine. It is to be noted that other reports which seem contradictory to the current outcome could be due to the difference in exposed compound and duration. For instance, reports suggested by Jurczuk et al., (2004), indicate an elevation in SOD and CAT as a consequence of cadmium toxicity. This could be due to the property of the chemical toxicant and is mainly based on the nature and concentration of the chemical compound which inflicts toxicity and additionally is dependent on the life stage of the exposed organism (Alvarez et al., 2006).

Glutathione peroxidase (GPx) belongs to a family of phylogenetically related enzymes and have been known to catalyze the reduction of H_2O_2 or organic hydroperoxides to water or the corresponding alcohols, respectively, typically using glutathione (GSH) as reductant (Dayer et al. 2008). Evaluation of GPx in renal tissue of exposed rats suggested irregular trend in its activity with a decline of -51.49 and -68.95 % was recorded for E2 and E3 respectively, with an elevation of 6.68% in E1. The trend observed in the current investigation could be due to the excessive build-up of free oxyradicals in the form of hydroperoxides as observed previously by Sharma et al., (2014).

Malondialdehyde (MDA) apart from being a major end product of LPO, is often thought to reflect the intensity of cellular injury within the organism exposed to environmental contaminants (Møller and Loft, 2010). Evaluating MDA levels helps in validating the intensity of damage to biological membranes (David and Kartheek 2016). In the present study, significant (*p<0.01) elevation in LPO levels were observed in all exposure groups as compared with group C. While E3 indicated the highest MDA levels which was found to be 296.42% as compared to control; E2 demonstrated lesser degree of damage as indicated by a percent change of 157.14 %. The third group E3 showed an elevation of 24.2 % which was also found to be significant, indicating the toxic insult of FPN on kidney in terms of oxidative stress.

A much stronger oxidant than superoxide anion-radical could initiate the chain oxidation of polyunsaturated phospholipids, thus leading to impairment of membrane Function (Klaunig et al., 2010). The current outcome indicates the damage in terms of structural aspect of kidney in rats exposed to FPN. Modifications of biomolecules by aldehyde products of LPO are also believed to contribute to lipofuscin formation, as seen in aging and in the progression of some degenerative diseases (Refsgaard et al. 2000). Lipids are considered to be one of the crucial biomolecules that are prone to be (Florens et al., 2016). Since kidney is an organ with abundance of long chain polyunsaturated fatty acids in the composition of renal lipids, its possibilities of being affected by oxidative stress are also known to be high (Vaziri, 2014). The present study indicated findings like damage to proximal and distal convoluted tubules within the nephrons of kidney of rats that were intoxicated with sublethal doses of FPN. The outcome might be due to the damage to the biomolecules in general and lipids in specific. Since, MDA is the principal and most likely outcome of polyunsaturated fatty acid breakdown, its persistence within biological system indicates and suggests the possible mechanism involved in the disintegration of renal tissue (Ozbek, 2012).

3.2 Histological analysis

The histopathological findings under C, E1, E2 and E3 of rat kidney are as given in Table 1 and Fig. 5. Changes in histoarchitecture indicated pathological outcome in kidney of exposed rats under all groups. The control rats however showed normal architecture with proximal and distal convoluted tubules and glomerulus. The function of the mammalian is to orchestrate the excretion of metabolic wastes found in blood, a function intimately related to its essential roles in general fluid homeostasis and osmoregulation (Scott and Quaggin, 2015). Changes in proximal and distal convoluted tubule structures as witnessed in exposed group of rats indicates the possibilities of complete obstruction of the urinary orifice by a sclerotic or crescentic lesion (Kriz and Le Hir, 2005). Tubular degeneration is observed under the conditions of deprived supply of filtrate delivery, initially resulting in inactivity atrophy with a progressive decomposition (Kaissling et al., 2013). The current outcome could therefore be attributed to the inadequate supply of filtrate to tubular structure that could in turn be due to the obstruction of urinary orifice as discussed earlier. The process of degeneration is thought to begin at proximal tubule segments under the vicinity of glomerulo-tubular junction and proceeds distally.

Role of kidney in coordination and maintenance of blood pressure and the promotion of erythrocyte development additionally has been previously discussed by Wadei and Textor (2012). Changes in the histoarchitecture suggests impairment in elimination potential of kidney which in present case is attributed to toxic possession of administered FPN. The outcome revealed damaged distal convoluted tubule with occurrence of vacuoles and tubular lumen under E1 group. The changes could be also as a consequence of persistent FPN within blood composite that is additionally broken down in to its metabolite which have been reported to be furthermore toxic than the parent compound (Simon-Delso et al. 2014). Filtration through the glomeruli and some degree of re-absorption in the tubules are essential features of renal activity. Structural damage to these units could therefore cause obstructions in blood flow resulting in hypoxia and cellular death (Yamamoto et al., 2006). These results are in agreement with the current findings as necrosis was witnessed under E3 group of rats suggesting dose dependent damage potential of FPN.

Studies by several authors have previously have implicated a wide range of environmental toxicants including pesticides and in impairing kidney functions (David and Kartheek, 2014). With occurrence of findings like proximal tubular nephropathy, glomerular sclerosis and interstitial fibrosis (Diamond, 2005). The findings observed in current study have been discussed previously wherein histopathological features of renal injury in humans, including intranuclear inclusion bodies and cellular necrosis in the proximal tubule and interstitial fibrosis have been the cause of failure in renal function (López-Novoa et al., 2011; Nogare et al., 2015). Degeneration of epithelial cells was observed as an important finding under current study. Changes in tissue architechture, with fibrosis was a clear outcome witnessed in E2 and E3 rats. Degeneration of epithelial cells with a characteristic feature of fibrotic tissue occurrence has been witnessed in E2 and E3 which could also be due to the obstructions in blood flow, this part of study derives the from previous reports by Basile et al., (2012).

4. CONCLUSION

The present outcome suggested the renal toxicity potential of FPN at the selected dose and duration. While the higher doses at E2 and E3 were found to distress the biochemical and histological factions, dose level of E1 was found to modulate the structural aspects only without altering the biochemical constituents significantly. The present work can be of crucial importance in providing a preliminary set of data as the literature on kidney damage for the selected toxicant is first of its kind. Thus from the outcome of current study it is recommended that care has to be taken whenever FPN is used under the proximity of mammalian class.

5. ACKNOWLEDGEMENTS

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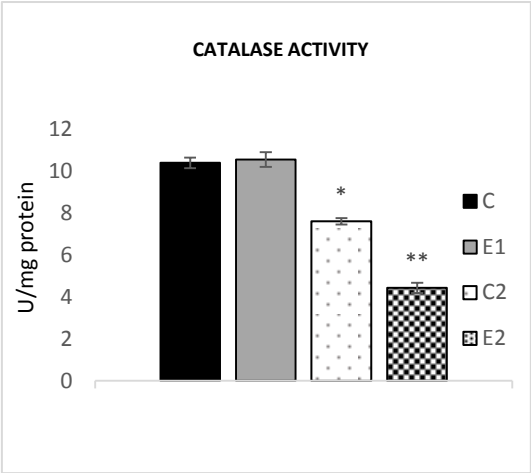

Fig. 1: Changes in renal catalase activity of rats exposed to sublethal doses of FPN

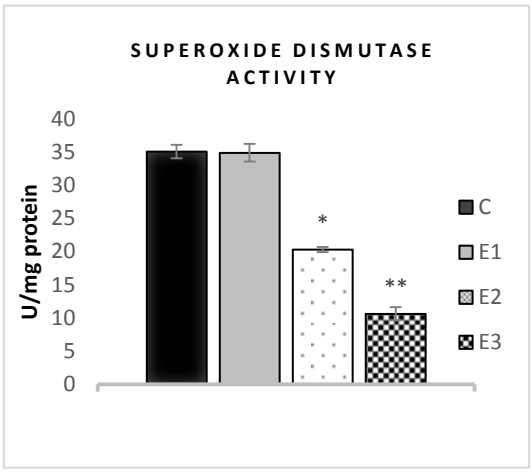

Fig. 2: Changes in renal superoxide dismutase activity of rats exposed to sublethal doses of FPN

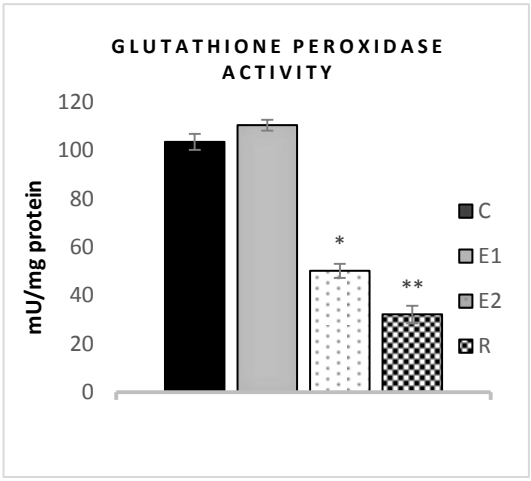

Fig. 3: Changes in renal glutathione peroxidase activity of rats exposed to sublethal doses of FPN

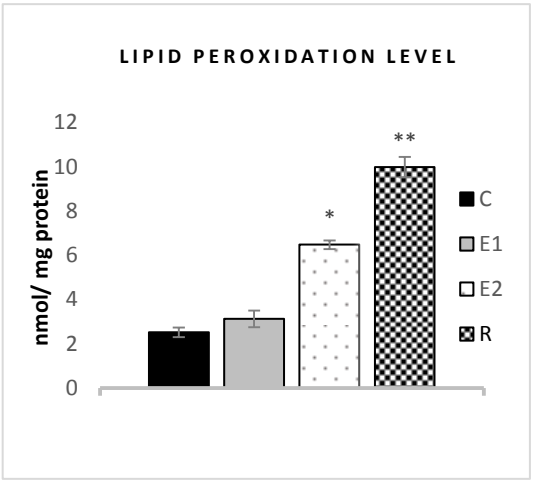

Fig. 4: Changes in renal malondialdehyde levels of rats exposed to sublethal doses of FPN

Table 1: Histopathological changes in kidney of male Wistar rats following oral administration to different doses of FPN

Findings	Groups			
	Control	Exposed		
	C	E1	E2	E3
Damaged Proximal Convoluted Tubule (PCT)	ND	* *	* *	*
Glomerulus degeneration (GD)	ND	* * * *	* * *	ND
Damage Distal Convoluted Tubule	ND	* *	* *	*
Glomerular Degeneration	ND	* *	* *	*
Degeneration of epithelial cells	ND	* * *	* *	*
Tubular lumen (TL)	ND	* * *	* *	ND
Blood coagulation (BC)	ND	*	* *	ND
Necrosis (N)	ND	* * * *	* * *	ND
Glomerular space (GS)	*	* *	* *	* *
Tissue damage (DT)	ND	* *	* * *	* * * *
Vacuolation (V)	ND	*	* *	* * * *
Fibrosis (F)	ND	ND	* * *	* * *

Histopathological findings in kidney of male Wistar rats with different groups indicated,
(ND) as not detected, (*) as low, (* *) as medium, (* * *) as high and (* * * *) as severe histoarchitectural damage.

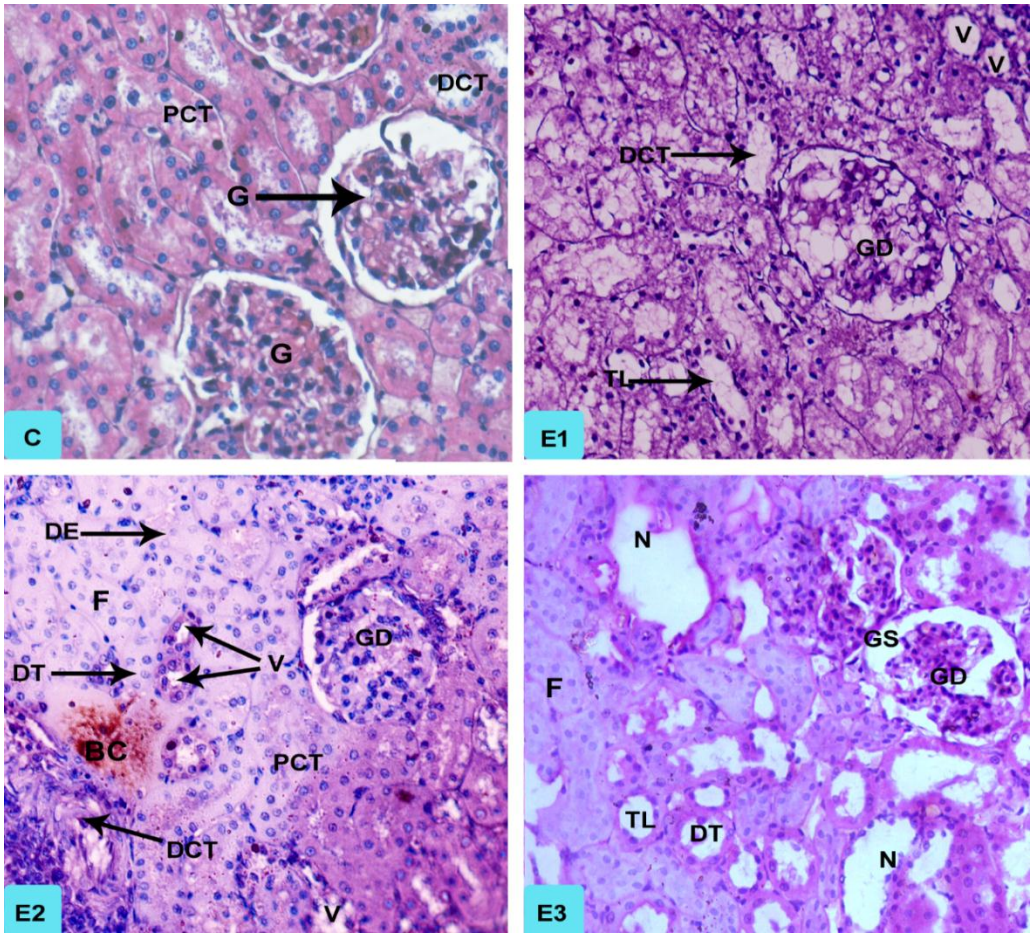

Fig. 5: Photomicrograph showing sections of rat kidney of male Wistar rats with histopathological findings following 90 day exposure to 0.0 (C), 6.46 (E1), 12.12 (E2) and 32.33mg/kg body weight (E3) of FPN (H and E staining, 200 X).

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