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STUDIES ON THE EFFECT OF FIPRONIL ON KIDNEY IN ALBINO RAT, *RATTUS NORVEGICUS*

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Abstract: Fipronil is a common phenylpyrazole insecticide with strong neurotoxic and systemic action. The present study explores its nephrotoxic effect on male albino rat *Rattus norvegicus*. Sixteen rats were grouped as control, low dose (10 mg/kg BW), and high dose (60 mg/kg BW) and were given fipronil orally for 21 days. Findings revealed a dose-dependent reduction in body and kidney weights. Histopathological analysis disclosed extensive renal injury in treatment groups with glomerular atrophy, tubular degeneration, mesangial enlargement, and vascular disruption. Biochemical profiles revealed marked reductions in kidney tissue protein, carbohydrate, DNA, and RNA content. Increased blood urea nitrogen and serum creatinine further testified to deranged renal function. These results indicate that fipronil causes extensive structural, biochemical, and functional changes in the kidney through oxidative stress and impairment of transport and metabolic processes. The damage was more intensive with the higher dose, indicating definite dose dependent nephrotoxicity. The findings from this research highlight the possible health hazards from long term exposure to fipronil and the need for careful use of such pesticides to avoid renal impairment.

Keywords: Fipronil, Nephrotoxicity, Oxidative stress, Serum creatinine, Blood urea nitrogen

1. Introduction

Fipronil is a broad-spectrum systemic insecticide belonging to the phenylpyrazole (fiprole) chemical group. It primarily targets insects by disrupting their central nervous system through interference with gamma-aminobutyric acid (GABA) regulated chloride channels, leading to neural hyperexcitation and death (Tingle *et al.*,2003). Due to its effectiveness, fipronil has been widely used since its classification by the U.S. Environmental Protection Agency (USEPA) in 1996 as a phenylpyrazole pesticide to replace organophosphates (Chiovarou and Siewicki, 2008). It is employed in various sectors including agriculture (e.g., seed and soil treatments), veterinary medicine (for pets like dogs and cats), and public health (Gupta and Anadon, 2018). Its lipophilic nature contributes to tissue accumulation, particularly in organs rich in fat content (Bhartiya *et al.*, 2020).

Fipronil has raised growing concerns due to its adverse effects on human and animal health, particularly in non-target organisms. It induces oxidative stress by generating reactive oxygen species (ROS), which disrupt cellular antioxidant systems and lead to mitochondrial damage, apoptosis, and tissue injury (Mossa *et al.*,, 2015; Uzunhisarcikli *et al.*,, 2023). Systemically, fipronil exposure has been linked to hepatotoxicity, neurotoxicity, nephrotoxicity, endocrine disruption, immunotoxicity, reproductive toxicity, and possible carcinogenic effects (USEPA, 1996; Abdel-Mobdy *et al.*, 2023). These toxicities are evident in various

biochemical and histopathological alterations across multiple organs, demonstrating the widespread impact of this compound.

Among the various organs affected by fipronil, the kidney holds particular importance due to its role in filtering blood, maintaining fluid and electrolyte balance, and eliminating waste products. The kidneys are composed of nephrons functional units that ensure efficient reabsorption and secretion processes critical to homeostasis. Studies have shown that fipronil can impair renal function, evidenced by elevated biomarkers like blood urea nitrogen (BUN) and creatinine, and histopathological signs such as tubular degeneration and glomerular damage (Sakr *et al.,* 2022; Uzunhisarcikli *et al.,* 2023). Investigating the nephrotoxic effects of fipronil is vital for assessing its impact on renal physiology and guiding protective strategies for environmental and occupational exposure.

Despite its agricultural and veterinary benefits, the broad and chronic use of fipronil presents potential risks that necessitate close toxicological scrutiny. Its ability to persist in the environment and accumulate in biological tissues heightens concern regarding prolonged exposure in both humans and animals. The compound's interference with vital physiological systems such as the nervous, hepatic, and immune systems emphasizes the importance of evaluating its safety profile. Understanding its mode of action and organ specific impacts is essential for developing regulatory policies and health interventions aimed at minimizing its adverse effects (Gunasekara *et al.,* 2007; Mohamed *et al.,* 2004; Kumar and Gopal, 2016; Simon-Delso *et al.,* 2015).

Due to scarcity of literature on fipronil induced nephrotoxicity the present study has been undertaken to investigate the histological, biochemical and functional aspect of kidney in fipronil treated albino rat.

2. Materials and Methods

2.1. Animal

The male albino rat, *Rattus norvegicus*, was selected as the animal model for this study due to its anatomical, physiological, and genetic similarity to humans. These rats are also cost-effective, safe, and easy to handle, making them a preferred choice for biological research.

2.2. Chemical

The chemical used in the study was Fipronil, purchased from BharatAgri Pvt. Ltd., Pune, India.

2.3. Animal Collection and Maintenance

Eighteen adult male albino rats (175–230 g) were procured from GLOBAL BIORESEARCH SOLUTIONS PVT. LTD., Nhuvi, Bhor, Pune (Reg. No. 1899/PO/RcNRcBt/5/16/CCSEA). The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and followed guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India (Reg. No. 478/G0/Re/S/01/CCSEA, dated 31st Oct 2016).

Animals were acclimatized for 7 days under standard laboratory conditions in cages with stainless steel lids. They were divided into three groups (A, B, and C), each containing six animals. Rats were fed a commercial pellet diet and provided water ad libitum. No special lighting was used beyond natural light, and room temperature was maintained at 26–28°C.

2.4. Treatment

Fipronil powder, obtained from BharatAgri Pvt. Ltd. (Pune, India), was administered orally to experimental groups by mixing it in distilled water. The study involved three groups of rats: Group A served as the control and received only normal food pellets along with distilled water; Group B received a low dose of Fipronil at 10 mg/kg body weight/day; and Group C received a high dose at 60 mg/kg body weight/day. The treatments were administered daily for 21 consecutive days. At the end of the treatment period, the body weight of each rat was recorded. The kidneys were then dissected out, cleaned, weighed, and preserved for further histological and biochemical analysis.

2.5. Body Weight and Organ Weight

At the time of sacrifice, the body weights of all rats were recorded. Kidneys were dissected, cleaned, and weighed to determine their absolute organ weight.

2.6. Histology

After dissection, the kidneys were rinsed in buffer saline to remove residual blood and fixed in freshly prepared Bouin’s fixative. Following fixation, the tissues were dehydrated through a graded series of ethyl alcohol, cleared in xylene, and embedded in paraffin wax. Thin sections of 5 µm thickness were cut using

a microtome, mounted on clean glass slides, and stained with hematoxylin and eosin. The stained slides were examined under a light microscope for histopathological analysis.

2.7. Biochemical Techniques

2.7.1. Extract Preparation

Kidney tissues were dissected under ice-cold conditions using Ringer's solution, cleaned, and homogenized for 5 minutes. The homogenizing medium varied depending on the assay (Ringer's for proteins, distilled water for carbohydrates and nucleic acids).

2.7.2. Protein Estimation (Lowry's Method, 1951)

Protein content was measured using Lowry's method with BSA as a standard. Color intensity was read at 650 nm after reacting the extract with copper reagent and Folin–Ciocalteu solution.

2.7.3. Carbohydrate Estimation (Dubois' Method, 1956)

Carbohydrates were quantified by reacting kidney extracts with phenol and sulfuric acid. The resulting yellow-brown color was measured at 490 nm.

2.7.4. DNA Estimation (Burton's Method, 1956)

DNA levels were estimated using the diphenylamine reaction, producing a blue color measured at 500 nm. Calf thymus DNA was used as the standard.

2.7.5. RNA Estimation (Orcinol Method, 1955)

RNA was measured using the Dische–Orcinol method. The green color developed was read at 660 nm, with yeast RNA serving as the standard.

2.8. Kidney Function Test

To assess renal metabolic function, blood urea nitrogen (BUN) and serum creatinine levels were measured, as they are established indicators of kidney performance. Raised levels generally suggest impaired filtration and metabolic disturbance. In the present study, blood samples were obtained from both control and treated groups to evaluate any treatment-related changes. The analyses were carried out using enzyme-linked immunosorbent assay (ELISA) kits, chosen for their high sensitivity and accuracy. All procedures were conducted at Bio Chem Lab, Nagpur.

2.9. Statistical Analysis

The observational data for all parameters were presented as individual values as well as mean ± standard error (SE). Differences were considered statistically significant at $p < 0.05$. Statistical analysis was performed using GraphPad software (2024 version).

3. Results

3.1. Body weight and organ weight

The results of the present study indicated an increase in body weight in the control group, while rats treated with 10 mg/kg body weight of Fipronil showed a significant reduction. A more pronounced and highly significant decrease in body weight was observed in rats administered 60 mg/kg body weight of Fipronil. This suggests a dose-dependent decline in body weight among treated animals compared to controls. Additionally, kidney weight was reduced in rats treated with both 10 mg/kg and 60 mg/kg body weight of Fipronil over the 21-day treatment period (Shown in Table No.1).

Table 1: Showing Body Weight of animals and Weight of Kidney of control and treatment of different doses of FPN in albino rat for 21 days duration

Group and dose	Duration	No. of animals	Initial BW (gm)	Final BW (gm)	Weight of kidney (left) (mg)	Weight of kidney (right) (mg)
Group I (Control) Normal Saline	21 Days	6	216.67±6.67	256.67±6.67	0.815± 0.00216	0.816±0.00216
Group II (Low dose) 10mg/kg BW	21 Days	6	224.53±1.33	201.67±3.33**	0.726± 0.00187*	0.695±0.00187**
Group III (High dose) 60mg/kg BW	21 Days	6	231.67±3.33	196.67±1.33***	0.692±0.00192***	0.585±0.00239***

3.2. Histology

In this study, the kidneys of control male albino rats exhibited normal histological features. The structures—including well-defined distal convoluted tubules (DCT), glomeruli (G), Bowman's capsules and spaces (BC and BS), proximal convoluted tubules (PCT), mesangial cells (M), and renal tubules (RT)—were clearly visible, active, and typically arranged in organized clusters.

In contrast, rats exposed to Fipronil at a dose of 10 mg/kg body weight for 21 days showed marked histological changes. These included expansion of mesangial regions, thickening of the basement membrane, dilated blood vessels in both the cortical and medullary regions, disruption of the endothelial lining, inflammation within the glomeruli, and degenerative changes in key renal structures such as the RT, PCT, and DCT.

Even more pronounced damage was observed in rats treated with a higher dose of Fipronil (60 mg/kg body weight) for the same duration. Their kidneys exhibited extensive pathological alterations, including ruptured renal tubules, narrowed blood vessels, glomerular degeneration, and frequent deterioration of the DCT, PCT, and mesangial cells (Shown in Figure No.1).

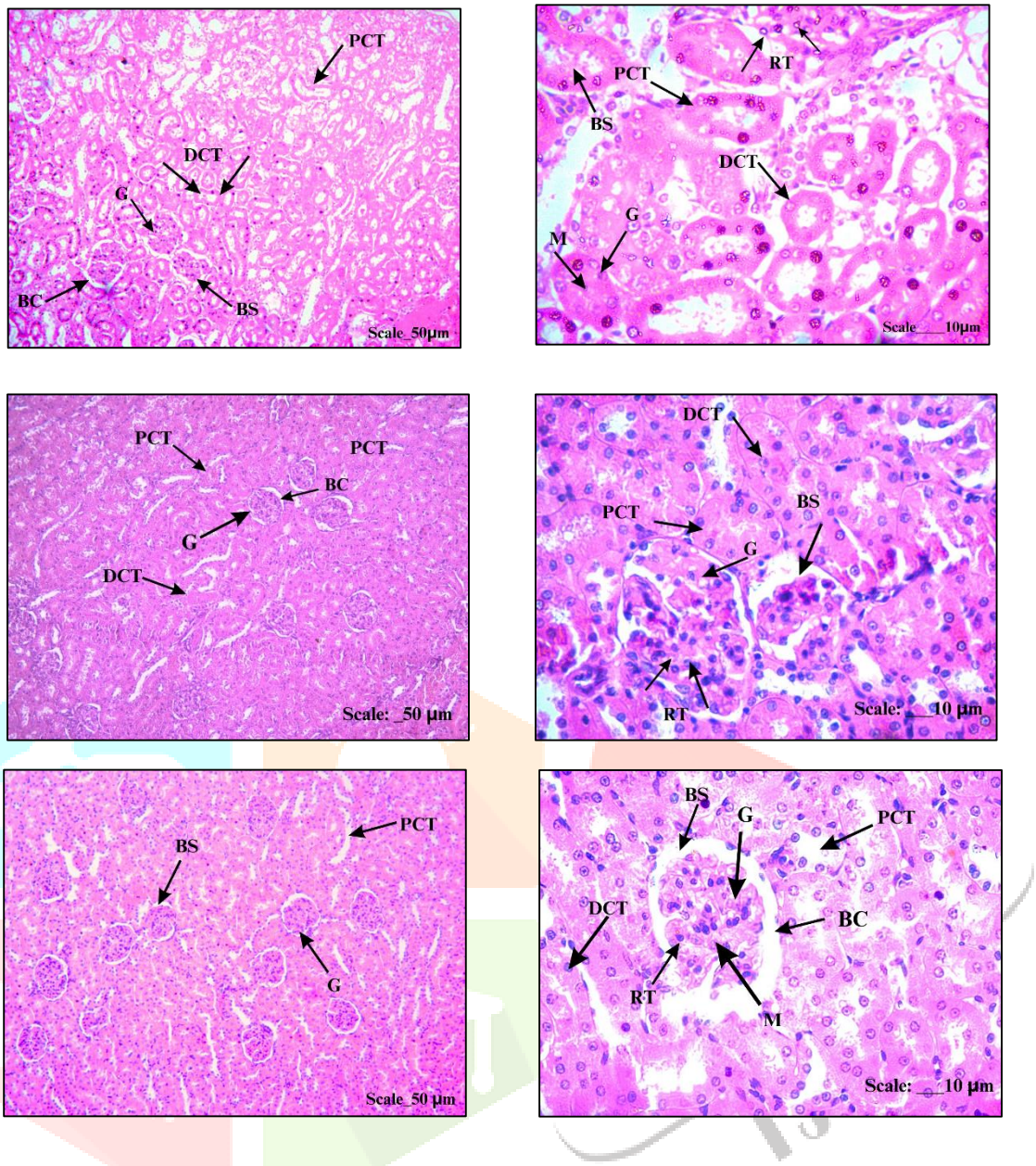

Figure 1. Photographs of transverse section of kidney in control group, 10 mg/kg BW and 60 mg/kg BW for 21-days duration in male albino rat (Abbreviations used: G- Glomerulus, PCT- Proximal Convoluted Tubule, DCT- Distal Convoluted Tubule, BC- Bowman's Capsule, BS- Bowman's Space, RT- Renal Tubule, M- Mesangial Cells)

3.3 Biochemical Estimations

3.3.1. Protein Estimation

In the control group administered saline, the protein concentration in kidney tissue was recorded as 261.52 ± 0.0117 µg/mL. A marked reduction was observed in rats treated with a low dose of Fipronil (10 mg/kg body weight), with protein levels decreasing to 243.66 ± 1.763 µg/mL. This decline was even more pronounced in the high-dose group (60 mg/kg body weight), where protein concentration dropped significantly to 173.24 ± 1.2602 µg/mL compared to the control (Table No.2).

3.3.2. Carbohydrate Estimation

In the control group, the carbohydrate concentration in kidney tissue was measured at 105.47 ± 0.0292 µg/mL. Treatment with a low dose of Fipronil (10 mg/kg body weight) resulted in

a considerable decrease to 80.46 ± 0.2302 µg/mL, while exposure to a high dose (60 mg/kg body weight) led to a further significant reduction, with levels dropping to 68.54 ± 0.3050 µg/mL (Table No. 2).

3.3.3. DNA Estimation

The concentration of DNA in the kidney tissue of male rats exposed to 10 mg/kg body weight of fipronil showed a noticeable decrease, while a marked reduction was observed in the high dose group (60 mg/kg BW), as compared to the control group (Table No.2).

3.3.4. RNA Estimation

The RNA concentration in kidney tissue decreased significantly in both treated groups. The low dose group showed a reduction, and a significant decline was recorded in the high dose group, as compared to the control group (Table No.2).

Table 2: Showing Protein, Carbohydrate, DNA and RNA concentration in Kidney of control and treatment of different doses of FPN in albino rat for 21-days duration

No. of animal	Treatment	Protein Concentration in kidney (mg/100mg)	Carbohydrate concentration tissue (Kidney) (mg/100mg)	DNA Concentration in kidney (mg/100mg)	RNA concentration in Kidney (mg/100mg)
6	Saline water	261.52±0.0117	105.47± 0.0292	0.334 ± 0.0250	0.311 ± 0.0012
6	Low dose (10mg/kg BW)	243.66±1.763*	80.46± 0.2302**	0.221 ± 0.0176**	0.239± 0.0082**
6	High dose (60mg/kg BW)	173.24±1.2602***	68.54±0.3050***	0.177±0.0048***	0.164±0.0025***

3.4. Serum Biochemical Analysis of Blood Urea Nitrogen level and Serum Creatinine Level

The blood urea and serum creatinine levels were assessed to evaluate kidney function across different treatment groups. Rats administered with a high dose of fipronil showed a significant increase in blood urea levels compared to the control group along with serum creatinine levels were significantly elevated. The low-dose group showed a slight increase in blood urea and a moderate increase in serum creatinine. These findings indicate a dose-dependent impact of fipronil on kidney function, with high-dose exposure impairing renal filtration capacity.

Table 3: Showing blood urea and serum creatinine concentration in Kidney of control and treatment of different doses of FPN in albino rat for 21 days duration

No. of animal	Treatment	Blood urea nitrogen (kidney) (mg/dl)	Serum Creatinine (Kidney) (mg/dl)
6	Saline water	13.50± 0.791	0.30± 0.158
6	Low dose (10mg/kg BW)	17.50 ± 0.3162	0.54± 0.0192
6	High dose (60mg/kg BW)	22.50±0.3162***	0 . 7 1 ±0.0158***

4. Discussion

In the present study, oral administration of fipronil for 21 days at doses of 10 mg/kg and 60 mg/kg resulted in dose-dependent alterations in both kidney structure and biochemical parameters. Treated rats showed significant reductions in body and kidney weights, likely due to oxidative stress, anorexia, and organ dysfunction. These findings align with those reported by Sharma *et al.,* (2018) and Khalil *et al.*, (2017), who observed similar weight loss and organ shrinkage following fipronil exposure.

Histopathological analysis confirmed structural damage in both liver and kidney tissues, with severity increasing at higher doses. Notable changes included glomerular atrophy, tubular degeneration, and inflammatory infiltration in the kidney, along with hepatocellular degeneration in the liver. These results support the observations of Sharma *et al.,* (2018) and Khalil *et al.,* (2017). Additionally, similar tissue degeneration has been reported in aquatic species exposed to fipronil, indicating its broad-spectrum toxicity (Nasr *et al.*,, 2021).

Biochemically, fipronil exposure significantly reduced total protein and carbohydrate content in kidney tissues, suggesting oxidative damage, impaired synthesis, and enzyme inhibition. These metabolic disruptions are consistent with previous findings by Khalil *et al.,* (2017) and Sarma *et al.,* (2020), and reflect fipronil's capacity to interfere with key physiological functions.

Markers of genotoxicity were also evident, as shown by significant DNA fragmentation and decreased RNA levels in kidney tissues. These alterations likely stem from oxidative damage and reduced transcriptional activity, corroborating the studies by Yousef *et al.*, (2021) and Khalil *et al.,* (2017), which highlighted nucleic acid degradation post-fipronil exposure.

Finally, significant elevations in blood urea nitrogen (BUN) and serum creatinine levels were observed in treated rats, indicating impaired renal function. These biochemical indicators, aligned with histopathological changes, point to fipronil-induced nephrotoxicity. Similar findings were reported by Elgawish and Abdelrazek (2014), further reinforcing concerns regarding fipronil's impact on renal health.

The research proved dose-related reduction of body and organ weights and gross histopathological changes in kidney tissues. Increased serum levels of urea and creatinine substantiated renal impairment. Decreases in the levels of protein, carbohydrates, DNA, and RNA indicate interference with metabolic and genetic processes. These results indicate the possible dangers of long-term Fipronil exposure and the importance of careful usage of such pesticides.

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During the preparation of this work, the author(s) used (Grammarly) in order to (construct heavy grammatically correct sentences and paraphrasing). After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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