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Research Article

Comparative Histopathological Evaluation of Deltamethrin-Induced Nephrotoxicity and the Biosafety of *Ocimum sanctum* Leaf Extract in *Rattus norvegicus*

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ABSTRACT

The purpose of this study was to assess the histopathological effects of deltamethrin and a plant-based bioactive extract from Ocimum sanctum (Tulsi) on the normal kidneys of Albino rats. Deltamethrin, a Type II pyrethroid insecticide, is widely used for pest control. Thirty rats were divided into three groups (control and two experimental), with 10 rats in each group. Group A (control group) rats received a normal diet; group B rats were treated with Tulsi leaf extract (0.5ml), and group C rats were administered a 0.5 ml dosage of deltamethrin (30 mg/kg) per day for five days. No histopathological changes were observed after administration of Tulsi leaves extract in renal tissues of group B and group A rats. Group C rats showed significant nephrotoxic effects, including necrosis and inflammation of renal tubules, degeneration of renal corpuscles and proximal tubule epithelium. Tubular lumens were enlarged and debris-filled, with dilated renal veins and mild glomerular congestion. These findings suggest that Ocimum sanctum is non-toxic to renal tissue and may possess protective antioxidant properties, supporting its potential role in mitigating pesticide-induced renal damage. Deltamethrin causes renal oxidative stress via excessive ROS generation, while Ocimum sanctum exerts nephroprotective effects through its antioxidant properties.

Keywords*: Synthetic insecticide, Deltamethrin, Tulsi leaf extract, Histopathological Alterations, Necrotic Agglomeration, Vacuolar Deterioration, Antioxidant Property.*

INTRODUCTION

The Lamiaceae family includes the fabled scented herb *Ocimum sanctum*. *O. sanctum*, also known as "The Queen of Herbs" (Bariyah, 2013). It is an upright, 30– 60 cm tall plant that has been used by people for more than 3000 years (Pattanayak et al., 2010).

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This medicinal plant has also been demonstrated to lower blood glucose levels, making it a successful diabetic treatment (Panchal & Parvez, 2019). It is used as a treatment for different diseases like malaria, the treatment of bronchitis, chronic fever, bronchial asthma, diarrhoea, dysentery, arthritis, skin infection, and painful infection of the eye (Modak et al., 2007). Pharmacological effects of Tulsi, which ease the person's chemical, metabolic, and psychological stress (Mohan, Amberkar, & Kumari, 2011). Tulsi shields the body against physical stress as well as chemical stress brought on by exposure to metals and industrial toxins (Ravindran et al., 2005). Tulsi (*Ocimum sanctum*) was chosen for this study because of its known health benefits (Cohen, 2014). It has antioxidant, anti-inflammatory, and kidney-protective qualities (Pattanayak, Behera, Das & Panda, 2010). Ocimum sanctum leaf extract significantly mitigates renal pathological alterations, indicating its protective effect against mercuric chloride-induced kidney damage (Sharma, Kumar & Kumar, 2005).

A type II pyrethroid known as deltamethrin is used all over the world to combat pests that spread disease and harm crops (Jyothimol et al., 2014). For fish, deltamethrin is hazardous (Haverinen & Vornanen, 2016). It is nevertheless neurotoxic and also an allergen that some people develop asthma from (Seery et al., 2003). Moreover, there is a strong link between toxicity and oxidative stress and metabolism (Wang et al., 2016). Synthetic insecticides also discovered in the nephrotoxicity caused by deltamethrin were apoptosis and oxidative stress signaling (Küçükler et al., 2021). In the liver, kidney, and lungs, deltamethrin was discovered to cause histological changes (Manna et al., 2005).

This study holds significance for the scientific community, particularly in the fields of toxicology, pharmacology and environmental sciences, as it addresses the growing concern over pesticide-induced toxicity and explores potential natural remedies (Soderlund et al., 2002; Mossa, Mohafrash & Chandrasekaran, 2018). Deltamethrin causes renal injury through ROS overproduction, lipid peroxidation, mitochondrial dysfunction, and depletion of endogenous antioxidants, resulting in tubular necrosis and glomerular damage (Smith et al., 2018; Sharma & Singh, 2019).

The kidney is a large, bean-shaped organ that is the main organ of the urinary system. Depending on the specific species, it may be elongated, spherical, or irregular (Baranski, 2023). The kidneys are situated near the spine, below the ribs (Sampaio, 2000). The right kidney is near the colon, duodenum, and liver (Lopez, Gogna & Khorasani-Zadeh, 2018). The left kidney is near the colon, pancreas, stomach, and spleen, held in place by ligaments (Soriano, Penfold, & Leslie, 2018).

The kidney is the most important organ for maintaining the internal environment of the human body by regulating the blood pressure, pH, composition of blood, and acid-base balance of the body (Hall et al., 2010). The kidney plays a significant role in blood filtration, reabsorption of essential substances and excretion of various metabolic waste products (Rostoka et al., 2025). The kidneys receive 20% of cardiac output for blood filtration, with self-regulating blood flow through specialized vessels in the glomeruli capillaries (Wallace, 1998).

The main objectives of this project are to investigate the histological alteration in the kidney caused by Tulsi leaves extract and synthetic deltamethrin. It focused on understanding the toxicity and safety of both substances in a healthy system. The goal was to demonstrate that Tulsi extract does not harm kidney tissues, unlike deltamethrin, positioning Tulsi as a safer choice than synthetic insecticides (Parveen, Perveen, Ahmad, Naz & Riaz, 2023). It promotes the concept of reducing or replacing harmful synthetic insecticides with natural, plant-based options that are safer for both the environment and public health (Yousef et al., 2006). It emphasizes how important these natural alternatives are for farming and protecting the environment (El-Demerdash, 2011). The main aim of this study is to create natural, plant-based options instead of synthetic chemicals like deltamethrin (Dagar & Ramakrishna, 2024). Additionally, the nephroprotective role

of Ocimum sanctum is described through its flavonoid- and eugenol-mediated antioxidant and membrane-stabilizing effects that protect renal tissue against oxidative stress (Prakash & Gupta, 2005; Pattanayak et al., 2010).

MATERIALS AND METHODS

Rearing of rat colonies

Thirty male Wister rats weighing between 150-200g were taken into the cages from the animal house of the University of Punjab under controlled laboratory conditions, such as 23-25°C room temperature, regular access to water and food for a 12-hour dark and light cycle. All rats were acclimatized for one week and divided into three cages, containing 10 rats each. These three groups of rats were named as the control group (Group-A), the positive control group (Group-B) and the negative control group (Group-C) or the experimental groups.

Collection and Preparation of insecticides

Plant-based bioactive extract from *Ocimum sanctum* (Tulsi) leaves was collected from the botanical garden of the University of the Punjab, Lahore. Fresh leaves of *Ocimum sanctum* (Tulsi) weighing 20g were properly cleaned with distilled water twice. All leaves were cut into small pieces after washing and mixed in 100 ml of distilled water. The mixture was allowed to boil for 5 minutes and filtered with the help of Whatman Filter paper after cooling. The formulation of deltamethrin utilized was 1.25% Deltamethrin ULV from Gharda Chemicals in Mumbai, India.

Dose administration

Group-A control group rats were treated with 0.5ml of 0.9% saline water, 0.5 ml of Tulsi leaves extract was given orally to the Group-B rats in 18 accordance with their body weight once per day for 5 days in the morning. Group-C rats were administered a dose of 0.5ml of deltamethrin orally according to their body weight for five days, once per day at 10:00 A. M. Animals were weighed before and after being injected with different doses.

Dissection of animals

Animals were given anaesthesia during dissection with the help of intraperitoneal injection of a mixture of distilled water and Ketamine. For maintaining the sterile condition, 70% ethanol was sprayed on the skin of all rats. Dissection was done by using sharp scissors to cut through the center of the middle region of rats to reveal their internal organs for the surgical removal of the kidneys.

Collection of tissue samples

The kidneys of rats were placed into the petri dish containing 0.9% saline water and cut into small slices. These small sections of the kidney were placed and preserved in 10% formalin containing labelled glass vials for further processing.

Tissue processing

Rat kidneys were dissected, cut into small pieces, and fixed in 10% formalin for 24 hours to prevent microbial deterioration. After washing with tap and distilled water, tissues were dehydrated through ascending concentrations of ethanol. Absolute alcohol steps (100%) were used to complete the dehydration process. Tissues were cleared in xylene and infiltrated with molten paraffin at 60°C over 24 hours. Cleared tissues were embedded in clove oil–greased molds, positioned, and solidified into paraffin blocks. Excess wax was trimmed, and blocks were mounted on wooden supports for further processing. Embedded tissue blocks were trimmed and sectioned using a microtome, and intact ribbons were collected while initial, incomplete sections were discarded. Ribbons were floated on a warm water bath to remove wrinkles and excess wax, then transferred onto slides. Slides were dried in a hot air oven and stored at 4°C for future use. Mayer's adhesive was prepared by mixing 50% egg albumin and 50% glycerol with a pinch of thymol, applied as a thin film on slides, oven-dried, wrapped, and stored at 4°C until use. Tissue sections were deparaffinized, rehydrated, and stained with hematoxylin and eosin, followed by dehydration and clearing. Slides were permanently mounted with DPX and Canada balsam for microscopic examination at low and high magnifications.

Microscopy & Photomicrography

These prepared histological slides were observed under low and high magnification of the microscope. Renal tissue section slides were examined microscopically under different magnifications of microscopes (10X, 40X), and representative photomicrographs were captured for detailed morphological analysis.

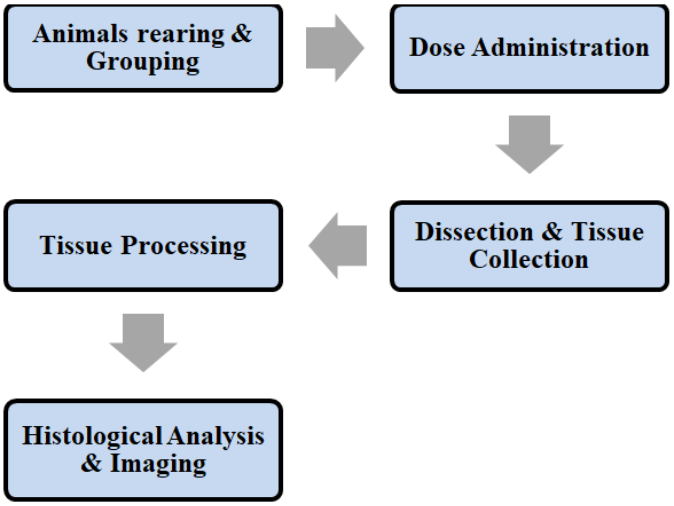

Figure 1. Experimental workflow and microscopic analysis

RESULTS AND DISCUSSION

Group-A

The control group contained normal rats that showed normal renal histology with two distinct regions: renal cortex and renal Medulla. Many other structures also showed normal histology.

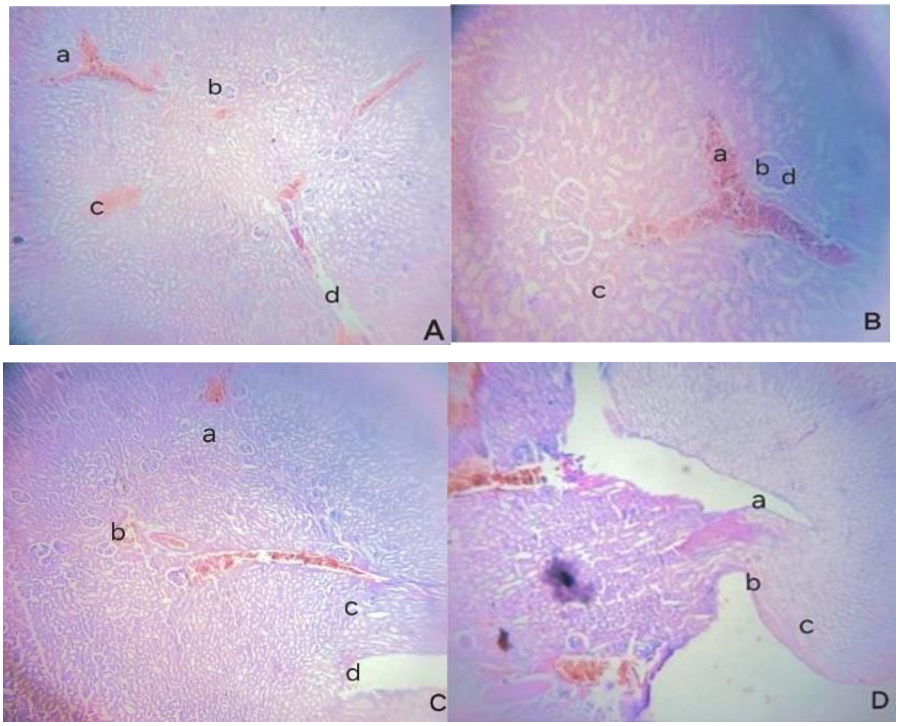

Figure 2: Hematoxylin and Eosin-stained kidney slide of the control group. A (a) Renal medulla, (b) Renal corpuscle, (c) Renal vein, (d) Collecting duct at 4x. B (a) Interlobular vein, (b) Glomerulus, (c) Proximal convoluted tubule, (d) Bowman's capsule at 40 X. C (a) Renal tubules, (b) renal arteries, (c) Renal papilla, (d) Columnar epithelium at 4X. D (a & b) Minor and major calyxes, (c) Renal cortex at 4X.

Group-B

Group B, also known as the experimental group or the Tulsi-treated group, rats. This group of rats was treated with the plant-based bioactive extract from Tulsi leaves (*Ocimum* sanctum). Due to the antioxidant nature of *Ocimum sanctum* extract, all the structures of the kidney displayed normal histology.

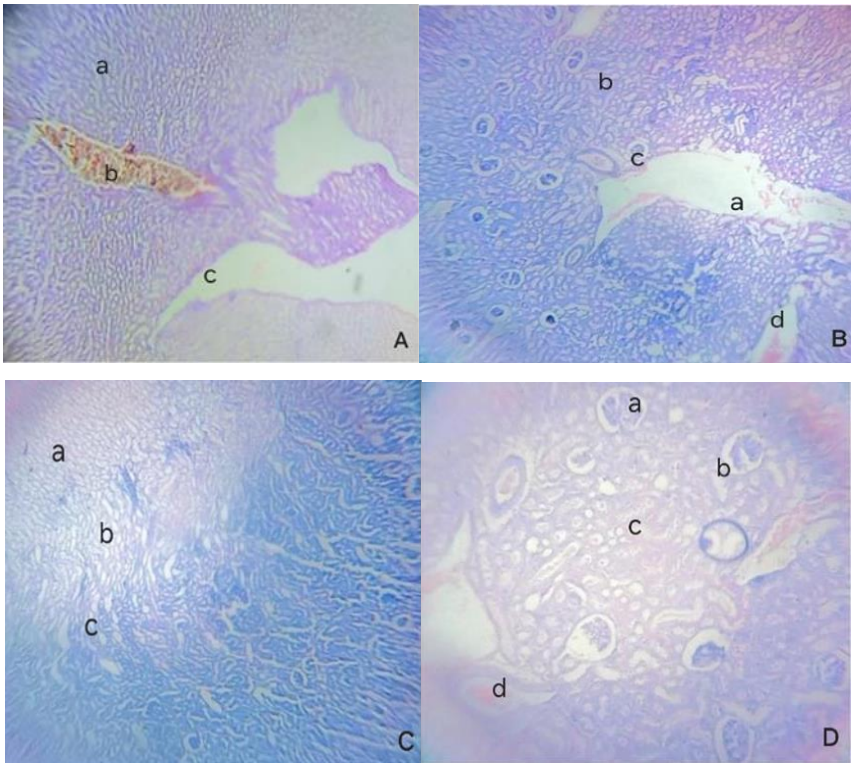

Figure 3: Hematoxylin and Eosin-stained kidney slide of Group B treated with *Ocimum sanctum* extract showed normal renal histology under low magnification. A, (a) Renal tubules, (b) Interlobular veins, (c) Minor calyx at 10X. B (a) Collecting duct, (b) Proximal convoluted tubules, (c) renal corpuscles, (d) Renal arteries and veins at 10X. C (a) Renal papilla, (b) Renal tubules, (c) Renal cortex at 10X. D (a) Glomerulus, (b) Renal corpuscles, (c) Proximal convoluted tubules, (d) arcuate arteries and veins at 40X.

Group-C

Group C, also known as the experimental group, was treated with deltamethrin. In this group, all rats were treated with deltamethrin (a synthetic insecticide), which induced different histopathological alterations in kidney architecture.

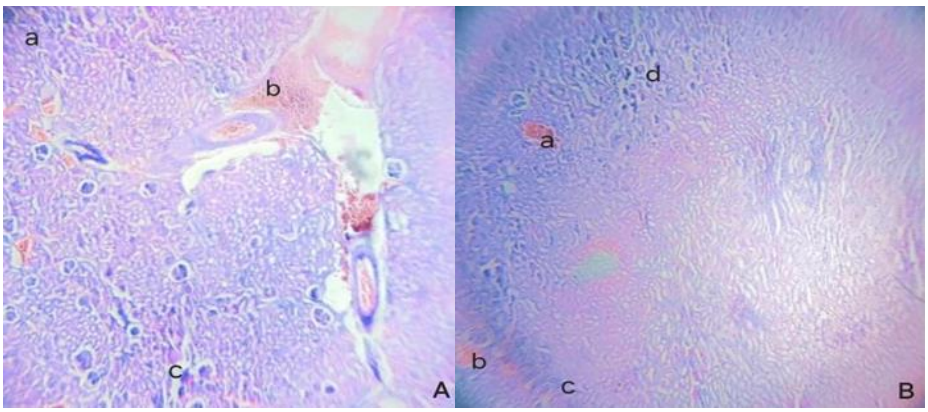

Figure 4.1: Hematoxylin and Eosin-stained kidney slide of Group-C rats treated with deltamethrin under low magnification. A, (a) Inflammatory cells or cellular debris, (b) Enlarged vein with glutted blood, (c) Dilated Bowman's capsule. B, (a & b) Congested arteries and veins, (c) Coagulative necrosis in tubules, (d) degenerated Glomeruli, inflammatory cells or cellular debris, at 10X.

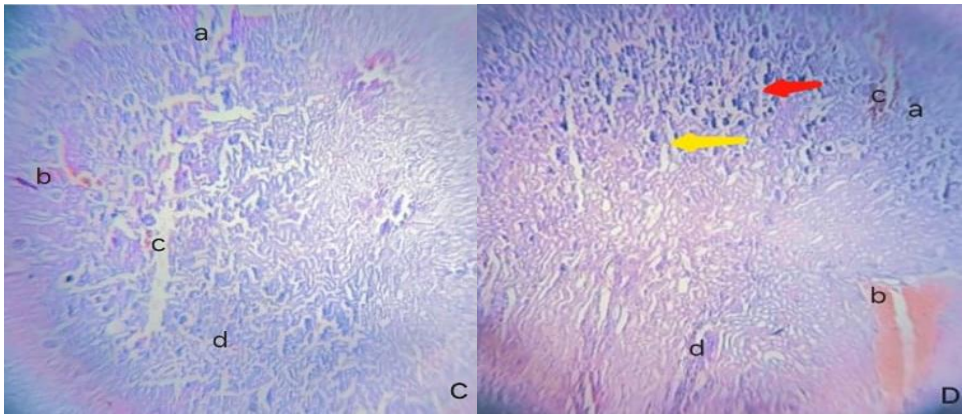

Figure 4.2: (a) Tubular atrophy, (b) Infiltration of WBC's, (c) Wide tubular lumen, (d) dilated renal tubule at 10X. C, (a) Damaged glomeruli, vacuolar degeneration of epithelial lining of proximal convoluted tubules (red arrow), cellular waste or enlargement in lumen of tubules (yellow arrow), (b & c) glutted blood in renal vein, (d) tubular necrosis or inflammation at 10X. D

DISCUSSION

The kidneys are subject to several histopathological changes caused by drug induced renal dosage (Radi, 2019). The kidney has a complicated structure, and any damage to that structure will affect the kidney (Schnellmann, 2001). The control group contained normal rats that showed normal renal histology.

Group B rats treated with the plant-based bioactive extract from Tulsi leaves showed no histological alterations in normal renal tissues. Tulsi (*Ocimum sanctum*) extract contains potent antioxidant compounds, including flavonoids and phenolics, that scavenge free radicals and upregulate endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase (Kelm et al., 2000; Mondal et al., 2009). These properties contribute to its nephroprotective effects by reducing oxidative damage and preserving the structural integrity of renal cells (Sakr & Al-Amoudi, 2012; Dubey & Shah, 2018). Supporting this, Sakr & Al-Amoudi (2012) reported that natural insecticides with antioxidant capabilities reduce deltamethrin-induced renal toxicity, acting as biosafety agents that preserve kidney structure and function. Ocimum sanctum extracts exhibit a high safety margin in rodents, showing no mortality or clinical toxicity. This supports the current study's finding of no overt renal or systemic adverse effects (Gautam & Goel, 2014).

In contrast, Group C rats exposed to the synthetic insecticide deltamethrin showed significant kidney damage, including glomerular degeneration, shrinkage, and the formation of cellular casts. These histopathological alterations are consistent with earlier studies by Sakr & Al-Amoudi (2012), who observed similar glomerular degeneration and Bowman's capsule dilation, and by Alarami (2015), who reported nephrotoxic effects from dimethoate, another synthetic insecticide. Deltamethrin disrupts renal redox balance by increasing lipid peroxidation (e.g., MDA) and depleting antioxidant defenses. This oxidative stress leads to cellular damage and glomerular degeneration (Chigrinski et al., 2018). The observed renal changes indicate that deltamethrin and similar compounds can lead to serious glomerular and tubular pathology. Collectively, these findings underscore the nephrotoxic potential of synthetic insecticides.

We also observed enlargement of the urinary space and Bowman's capsule, indicating glomerular damage. Haibo et al. (2011) reported similar changes with deltamethrin exposure, along with elevated BUN levels, suggesting renal impairment.

Our study revealed tubular degeneration and necrosis, along with vacuolar deterioration of the epithelial lining of proximal convoluted tubules in deltamethrin-treated rats, deltamethrin induces oxidative

stress through the generation of reactive oxygen species (ROS), which cause lipid peroxidation, cellular damage, and inflammation in renal tissues (Verma et al., 2013; Bortoli et al., 2015). In the same way, these findings are consistent with Choudhury and Ahmad (2006), who reported renal damage involving interstitial, tubular, and vascular cells following exposure to common drugs, leading to various renal syndromes.

Our study showed dilation and swelling of proximal convoluted tubules and enlarged tubular lumens in deltamethrin-treated rats. All these nephrotoxic effects were similar to those reported by Sun et al. (2022) in permethrin-exposed Albino rats.

CONCLUSION

The present study indicates that *Ocimum sanctum* is a biosafe agent with antioxidant properties and does not cause toxicity in the normal kidneys of rats. In contrast, deltamethrin induces nephrotoxicity and significant histological alterations in normal renal tissues. In future, this approach will enhance understanding by including biochemical parameters as well as impacts of both insecticides on other organs of rats to demonstrate how *Ocimum sanctum* protects against toxicity.

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Ethical concerns:

All experimental procedures were conducted in compliance with established guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the Local Ethical and Review Committee of the Department of Zoology, University of Education, Lahore.

Author contributions:

The authors sincerely acknowledge Fasiha Shamsher Ali for her valuable support and contribution. Fasiha Shamsher Ali, Fizza Javed, Afshan Syed Abbas, conceived and designed the research. Fasiha Shamsher Ali and Fizza Javed conducted the experiments. Fasiha, Taba Iftikhar, and Maleeha Shamsher prepared the first draft of the manuscript. Fasiha Shamsher Ali, Afshan Syed Abbas, Taba Iftikhar, Maleeha Shamsher, Fizza Javed and Javeria Gulzar helped in data analyses and reviewing the manuscript. All authors have approved the final version of the manuscript.

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Declaration of Competing Interest:

The authors declared that they have no competing interests.

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