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Nephroprotective Role of Withania Coagulans Fruit Extract Against Bifenthrin Toxicity: A Multimodal Analysis in Albino Mice

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ABSTRACT

Purpose: To evaluate the ameliorative effects of Withania coagulans extract against histopathological, micrometric and biochemical alterations in mice kidney exposed to bifenthrin, a fluorinated pesticide.

Methods: Forty male albino mice were divided into four groups: control (CG), W. coagulans treated (WT), bifenthrin treated (BT), and combined treatment (BWCT) with bifenthrin and W. coagulans extract. Mice were treated for 10 days, after which histopathological, micrometric, and hematological evaluations were conducted.

Results: Bifenthrin induced nephrotoxicity resulted in glomerular atrophy, tubular necrosis, inflammation, and vacuolization, with micrometric analysis showing decreased glomerular and tubular areas. Co-administration of W. coagulans extract ameliorated these effects, restoring kidney structure, reducing necrosis, inflammation, and vacuolization, and improving micrometric parameters. Hematological and renal biomarkers showed significant recovery to baseline levels.

Conclusions: W.coagulans has a nephroprotective role due to its ameliorative effects against the toxicity of bifenthrin among albino mice.

Keywords: *Bifenthrin; Nephrotoxicity; Hematology; Withania coagulans*

INTRODUCTION

Bifenthrin (C23H22ClF3O2) is a widely used synthetic pyrethroid insecticide, characterized by its trifluoro methylated structure that provides high hydro stability, photostability, and effectiveness against pests.[1] Among pyrethroids, it is the primary contributor to aquatic toxicity, causing both acute lethality and sublethal effects in non-target species.2 Bifenthrin, designated as a Class II toxicant by the US EPA[3] induces multi-system toxicity, including immunotoxic effects on human lymphocytes (notably T-cell impairment), developmental and neurobehavioral deficits, oxidative stress, endocrine disruption, and organ-specific damage to hepatic, pulmonary, renal, testicular, and neurological tissues.[4]

Studies suggest that exposure to pyrethroid in animals results in hyperglycemia and increased plasma levels of catecholamines (adrenaline and noradrenaline). The elevated catecholamines can increase cardiac output and vasoconstriction in the glomerular arterioles. The high level of catecholamines has also been associated with release of renin that in turn activates the renin-angiotensin system. The angiotensin II further promotes the release of vasopressin.[5-6] Bifenthrin induces nephrotoxicity by elevating renal oxidative stress, circulating cholesterol, lipid peroxidation, LDL, LDL-apoB 100, oxidized-LDL, the renal pro-inflammatory cytokines (TNF-α, IL-2, and IL 6) and generation of ROS (reactive oxygen species).[7] Natural antioxidants such as propolis, pollen, caffeic acid phenethyl ester and organoselenium compounds have shown strong antioxidant and tissue protective effects in rats.

Since ancient times plants have been utilized as traditional medicine due to their medicinal compounds.

W.coagulans, a member of Solanaceae family, is a renowned medicinal plant in traditional medicine distributed from the eastern Mediterranean region to South Asia. Research indicates that it possesses various pharmaceutical and phytochemical properties,[8] anti-inflammatory properties,[9] cardiotonic activities, hepatoprotective, antifungal,[10] hypoglycemic, free radical scavenging activity,[11] hypolipidemic,[12] wound healing properties, as well as antidiabetic nephropathy properties.[13] The dried ripe fruits of W. coagulans have traditionally been used for their antioxidant, anti-inflammatory properties, nephroprotective potential against diabetic nephropathy in rats. Withania coagulans is rich in antioxidants like withanolides, withaferin A, and coagulin which neutralize harmful reactive oxygen species (ROS) and reduce oxidative stress. Due to their anti-inflammatory and antioxidant properties, these compounds prevents inflammatory and oxidative stress-induced changes of cellular macromolecules and inhibit inflammation in chronic kidney disease. [14]

The fruit extract of W. coagulans was chosen in this study, aimed to explore the therapeutic potential of extract of W.coagulans (WCE) fruits to ameliorate the nephrotoxicity induced by Bifenthrin exposure in male Swiss mice.

MATERIAL AND METHODS

Research animals and their management

Forty mature male albino mice (Mus musculus 30–35 g), were housed in the animal facility of the Department of Zoology, University of Sargodha, under controlled environmental conditions (24–27°C, 55–65% humidity, 12-h light/dark cycle) with ad libitum access to standard rodent feed and water. All experimental procedures received prior approval from the Institutional Animal Ethics Committee (IAEC).

Animal treatment groups

Mice were subdivided into four groups as follows:

Control Group (CG)

Mice received saline and a toxicant free balanced diet from days 1 to 10.

Bifenthrin Treated Group (BT)

Bifenthrin was administered daily via drinking water (days 1–10) at a concentration delivering 5 mg/kg body weight/day. This dose was calibrated to an average water intake of 4.7 ml/day for a 30 g mouse. Dose solutions were prepared fresh daily by dissolving bifenthrin in vehicle. The selected oral dose corresponds to a dose proven to induce nephrotoxicity, such as oxidative stress, inflammation and kidney tissue damage, without causing severe systemic effects or death.[15]

W.coagulans (WCE) Treated Group (WT)

Mice received 0.1ml of pure Withania coagulans extract corresponding to an approximate dose of 333.33mg/kg body weight/day from day 1 to 10. The chosen oral dose of W.coagulans aligns within the range of 200-400mg/kg/day proved in rat studies to provide maximum nephroprotective effects of W.coagulans.[3]

Bifenthrin and W.coagulans combined treatment (BWCT) Group

Mice received 0.15ml Bifenthrin as well as 0.1ml of pure W.coagulans extract from the day 1 to day 10.

Preparation of Withania coagulans extract

Paneer Dodi (W. coagulans fruit) was purchased from the market. The aqueous extract of the W.coagulans fruit was prepared and stored in an airtight vial to prevent contamination.

Preparation of Bifenthrin Stock and Working Solution

A stock solution of bifenthrin (10.6% w/w) was prepared by dissolving 4.72ml of pesticide in 495.2 ml of distilled water. Afterward 9 mL of this stock solution was diluted with 300 ml of distilled water to create a working solution, which was administered at a dose of 0.1ml per mouse per day.

Body Weight Assessment

The body weights of mice were recorded at the onset of the experiment and at the end of the 10-day experimental period using a digital balance.

Animal dissection and blood collection

After the 10 days of study period mice were dissected. Blood samples were immediately collected from each group in blood collection tubes. These samples were further used for complete blood count (CBC) to investigate hematological parameters and to perform renal function tests to assess the levels of urea and creatinine.

Organ recovery

Following dissection, the kidneys of mice were removed. The weight of kidney of each group was also measured with the help of digital balance.

Preparation for Histopathological examination

The kidney specimens were fixed in acidified formyl ethanol for 48 hours to prevent degradation. Following standard histological protocols, the fixed tissues were embedded in wax, sectioned, and stained with Hematoxylin and Eosin to prepare permanent slides for examination.

Digital Imaging and Micrometric Analysis

Histological slides of kidney samples from each group were examined using a trinocular research

microscope at 10X and 40X magnifications. Images were processed further with ImageJ to enhance clarity, and labels were added for better understanding. For micrometric analysis, randomly selected sections of kidney were analyzed using CorelDRAW 11. The collected data were used to calculate group mean ± SEM values. Measurements included the mean number of podocytes, thickness of periglomerular space and cross-sectional area (CSA) of the glomeruli, PCTs and DCTs, using the formula:

$$CSA = (length \times width/4)\ \pi$$

Statistical Analysis

Data analysis was conducted using SPSS 20.0 software. Statistical significance was figured out through ANOVA, followed by post-hoc analysis using Tukey's HSD test. Results were considered statistically significant for $p < 0.05$ and insignificant for $p > 0.05$.

RESULTS

Histopathalogical findings

The histological sections of the control group displayed typical features of normal kidney tissue under microscopic examination. These sections included clearly identifiable glomeruli with normal periglomerular space and proximal and distal tubules. The endothelial lining of the Bowman's capsule was intact and there were no indications of apoptosis. No abnormalities in tissue structure were observed (Fig.1A and 1B).

The group treated with bifenthrin displayed several pathological changes in contrast to control group that include partial glomerular degradation, increased peri-glomerular space between glomeruli and Bowman's capsules, endothelial cell damage in bowman's capsule with excessive endothelial cell death, and intracellular oedema. Many defaced or contorted glomeruli with accumulation of tissue fluid in peri-glomerular spaces were also observed, especially in the juxtamedullary nephrons of BT group. Additionally, BT group also displayed shrinkage of glomeruli, mild hemorrhage, highly atrophied cortical glomeruli, and intraglomerular fibrosis with a simultaneous reduction of podocytes. The increase in cross-sectional area of proximal and distal convoluted tubules was also observed (Fig.1C and 1D).

The group treated with W.coagulans displayed normal glomeruli, slightly increased periglomerular space, intact endothelial lining of Bowman's capsule and slightly increased size of proximal and distal tubules. There were no signs of hemorrhage in WT group (Fig. 1E and 1F).

The bifenthrin and W.coagulans combined treatment group(BWCT) displayed ameliorative changes compared to the bifenthrin group as indicated by rehabilitation of structure of glomeruli, in terms of glomerular regeneration in the basal cortical and juxtamedullary regions. Partial recovery from glomerular atrophy and interglomerular migration of podocytes were also observed in the BWCT group. However, some signs of medullary hemorrhage were also visible. The BWCT group also exhibited the rehabilitation of proximal and distal renal tubules in terms of CSA of tubules (Fig.1G and 1H).

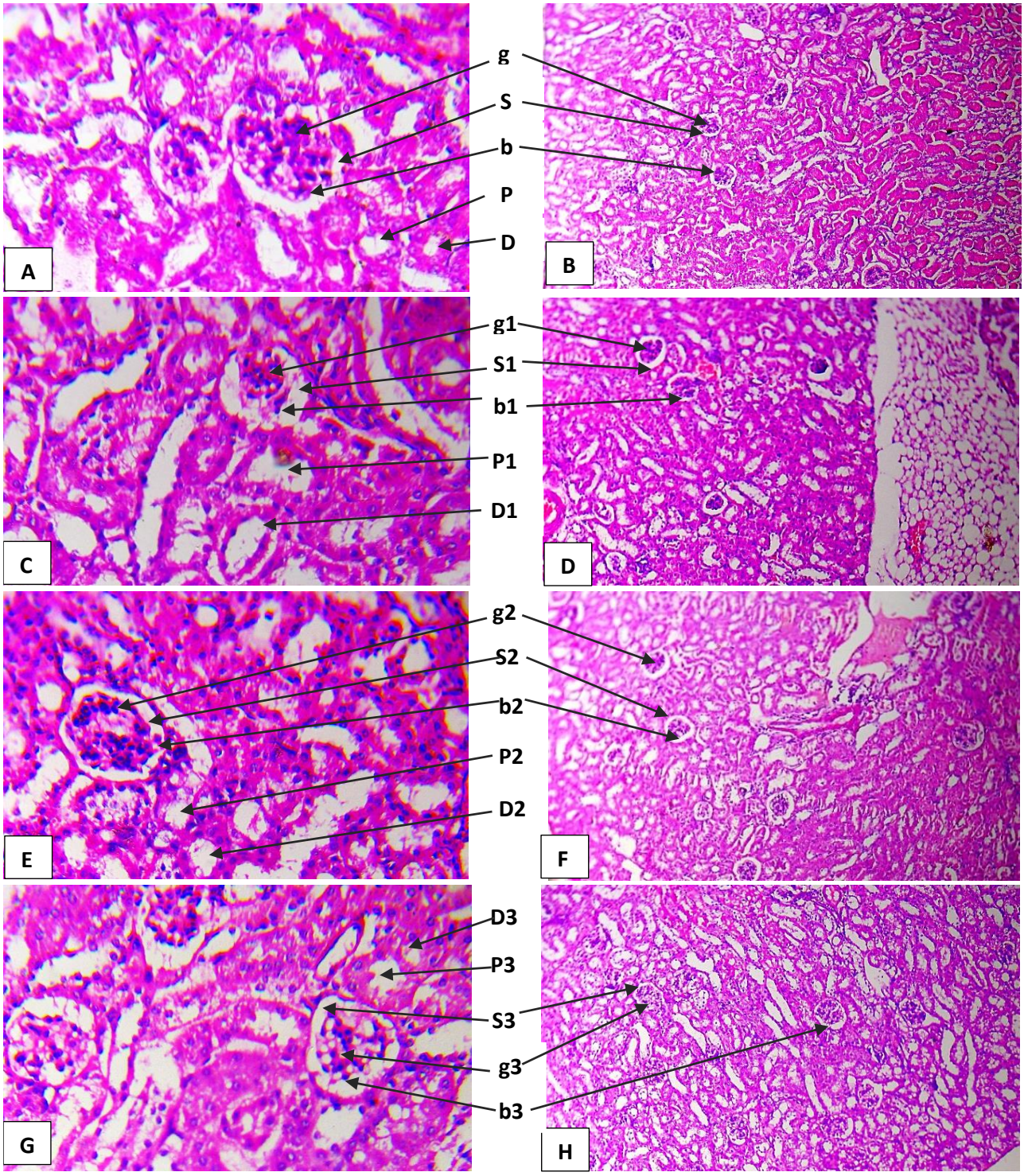

Figure 1. Microscopic images of a section from mice kidney A(CG), C(BT), E(WT) and G(BWCT) at 40x magnification and B(CG), D(BT), F(WT) and H(BWCT) at 10x magnification. G: glomeruli of normal size and shape, g1: shrunken glomerulus,g2: slight increase in glomerulus size, g3 rehabilitated glomerulus. S: Thin wall defined periglomerular space, S1: widened peri glomeruli space,S2: slightly increased periglomerular space , S3: recovered peri glomeruli space. B: Intact endothelial lining of bowman's capsule, b1: cell death in endothelial lining of bowman's capsule,b2: normal endothelial surface of bowman's capsule, b3: recovered endothelial layer of bowman's capsule, P:Proximal convoluted tubule of normal size , P1:Increased size of PCT ,P2: slightly increased CSA of PCT, P3: Rehabilitated PCT, D: Proximal convoluted tubule of normal size, D1: Increased size of DCT, D2: slightly increased CSA of DCT ,D3: Recovered distal convoluted tubule

Table 1. Comparative assessment of micrometric variations in four studied groups[1]

Micrometric Parameters	CG	BT	WT	BWCT	ANOVA (P-value)
Mean CSA of glomeruli (µm²)***	2112.46 ± 79.49^a	1143.87 ± 105.46^b	1883.71 ± 31.06^c	1759.93 ± 27.98^c	P ≤ 0.0001***
Thickness of periglomerular space (µm) ***	6.05 ± 0.15^a	10.37± 0.46^b	7.94 ± 0.33^c	8.49 ± 0.19^c	P ≤ 0.0001***
Mean number of podocytes /unit area (2500 µm²)***	34.30 ± 1.01^a	23.20 ± 0.48^b	31.30 ± 1.07^a	26.10 ± 0.76^b	P ≤ 0.0001***
Mean number of glomeruli /unit area (2500 µm²)**	5.90 ± 0.27^a	4.30 ± 0.21^b	5.40 ± 0.30^a	5.10 ± 0.17^a	*P* ≤ 0.001**
Mean CSA of PCTs (µm²)***	1091.70 ± 12.82^a	1293.00 ± 7.56^b	1102.80 ± 22.54^a	1217.00 ± 3.95^c	P ≤ 0.0001***
Mean CSA of DCTs (µm²)***	1094.00 ± 23.40^a	1272.50 ± 2.08^b	1094.50 ± 19.25^a	1208.70 ± 4.00^c	P ≤ 0.0001***

[1] Values are Mean ± SEM (n = 40) Groups sharing the different letter (a, b, c) are significantly different (P < 0.05).

Micrometric findings

Significant differences between the Control, BT, WT and BWCT groups were observed upon statistical analysis (Table2).

CSA of glomeruli: The control group recorded highest mean CSA of glomeruli (2112.46±79.49 µm²), followed by the WT group (1883.71 ± 31.06 µm²), BWCT group (1759.93 ± 27.98µm²), and the lowest in the BT group (1143.87 ± 105.46 µm²). Statistical analysis (ANOVA) showed a significant difference among the groups (P ≤ 0.0001). The post hoc analysis indicated a highly significant difference (P < 0.0001) between the BT and control group (mean difference = 968.59 µm², P < 0.001) , and between the BT and BWCT group (mean difference = 616.06 µm², P < 0.001) (Fig.2 a, Table 1).

Thickness of Periglomerular Space: The highest mean thickness of the periglomerular space was recorded in the BT group (10.37 ± 0.46µm), followed by the BWCT group (8.49 ± 0.19 µm), WT group (7.94 ± 0.33µm) and the lowest in the Control group (6.02 ± 0.15µm). Statistical analysis (ANOVA) showed a significant difference among four studied groups (P ≤ 0.0001). The post hoc analysis indicated a highly significant difference (P ≤ 0.0001) between the BT and control group (mean difference = 4.32µm, P < 0.001) and between the BT and BWCT group (mean difference = 1.88µm², P < 0.001) (Fig.2b, Table 1).

Number of Podocytes: The highest mean number of podocytes was recorded in the Control group (34.30 ± 1.01), followed by WT group (31.30 ± 1.07), the BWCT group (26.10 ± 0.76), and the lowest in the BT group (23.20 ± 0.48). Statistical analysis (ANOVA) showed a significant variation among four groups (P ≤ 0.0001). The post hoc analysis indicated a highly significant difference (P ≤ 0.0001) between the BT and Control group (mean difference = 11.10, P < 0.001) (Fig.2c, Table 1).

Number of Glomeruli: The highest mean number of glomeruli was observed in the control group (5.90 ± 0.27), followed by the WT group (5.40 ± 0.30),BWCT group (5.10 ± 0.17) and the lowest in the BT group (4.30 ± 0.21). Statistical analysis (ANOVA) indicated a highly significant difference among four groups (P < 0.001). The post hoc analysis showed significant difference between the BT and Control group (mean difference = 1.60, P < 0.001) (Fig.2d, Table 1).

CSA of PCTs: The highest mean cross-sectional area of proximal convoluted tubules was recorded in the BT group (1293.00 ± 7.56 µm²), followed by the BWCT group (1217.00 ± 3.95 µm²), WT group (1102.80 ± 22.54 µm²) and the lowest in the CG (1091.70 ± 12.82 µm²). Statistical analysis (ANOVA) showed a significant variation among the groups (P ≤ 0.0001). The post hoc analysis indicated a highly significant difference between the BT and Control group (mean difference = 201.30 µm², P < 0.001), and between BT and BWCT group (mean difference = 76.00 µm², P < 0.001) (Fig.2e, Table 1).

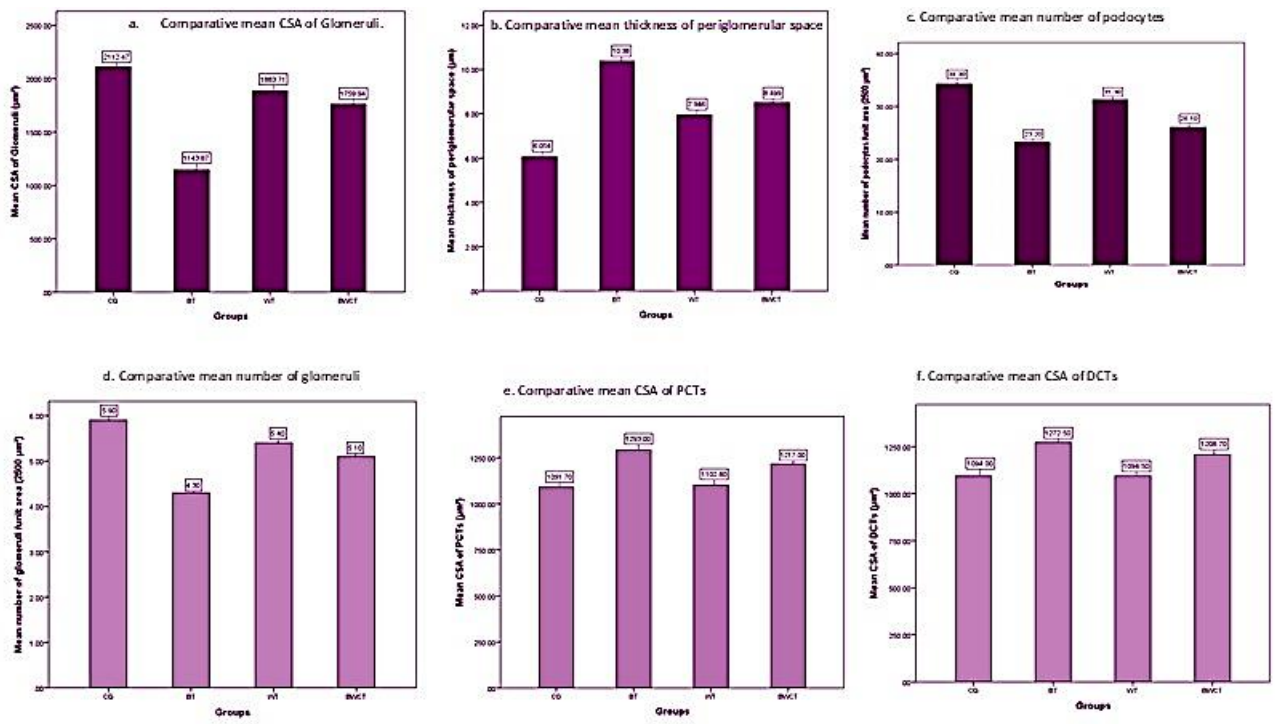

Figure 2. Comparative assessment of micrometric variations among study groups

Table 2. The comparison of mean values for serum urea and creatinine levels[2]

Parameter	CG	BT	WT	BWCT	ANOVA (P-value)
Urea (mg/dl) ***	18.46 ± 0.29^{a}	25.44 ± 0.37^{b}	21.68 ± 0.28^{c}	22.16 ± 0.37^{c}	P < 0.0001***
Creatinine (mg/dl)***	0.42 ± 0.01^{a}	0.75 ± 0.01^{b}	0.53 ± 0.01^{c}	0.65 ± 0.02^{d}	P < 0.0001***

[2] Values are Mean ± SEM (n = 40). Groups sharing the different letter (a, b, c, d) are significantly different (P < 0.05).

CSA of DCTs: The highest mean cross-sectional area of distal convoluted tubules was recorded in the BT group (1272.50 ± 2.08 µm²), followed by the BWCT group (1208.70 ± 4.00 µm²), WT group (1094.50 ± 19.25 µm²) and the lowest in the control group (1094.00 ± 23.40 µm²). Statistical analysis (ANOVA) showed a significant variation among the groups (P ≤ 0.0001). The post hoc analysis indicated a highly significant difference between the BT and Control group (mean difference = 178.50 µm², P < 0.001), and between BT and BWCT group (mean difference = 63.80 µm², P < 0.001) (Fig.2f, Table 1).

Renal function tests

The levels of serum urea and creatinine were highest in the BT group, followed by the BWCT group. ANOVA showed a significant variation (P ≤ 0.0001) in levels of urea and creatinine among four studied groups (Table 2)

Urea levels demonstrated a significant difference between the control group and BT group (P < 0.001). Serum urea levels were reduced by 12.89% (22.16 mg/dL) in the BWCT group compared to the BT group (25.44 mg/dL). Creatinine levels also differed significantly between the Control and BT group (P < 0.001). Serum creatinine levels were reduced by 13.33% (0.65 mg/dL) in the BWCT group compared to the BT group (0.75 mg/dL), WCE treatment reduced the levels of urea and creatinine demonstrating a protective effect of *W.coagulans* on kidney function.

Hematological findings

Exposure to bifenthrin caused significant hematological alterations. After bifenthrin exposure (10 days), significant decreases were noted in red blood cell (RBC) count, hemoglobin (Hb) percentage, mean corpuscular volume (MCV), Hematocrit(HCT) percentage, Mean Corpuscular Hemoglobin (MCH) and platelet count in BT Group. In contrast, mean corpuscular hemoglobin concentration (MCHC) increased significantly in the BT Group(Figure.4). The total leukocyte count (TLC), Lymphocytes percentage, Monocytes percentage and Eosinophils percentage also increased significantly. Treatment with WCE significantly restored these values aligning closely with control values (Table 3)

Table 3. Comparative assessment of Hematological variations in four studied groups[3]

Parameters	CG	BT	WT	BWCT
RBC (×10⁶/µl)***	8.99 ± 0.04ᵃ	6.46 ± 0.02ᵇ	7.39 ± 0.06ᶜ	7.53 ± 0.07ᵈ
TLC (×10³/µl)***	7.02 ± 0.05ᵃ	9.90 ± 0.09ᵇ	6.05 ± 0.02ᵃ	7.87 ± 0.14ᶜ
Neutrophils(%)***	13.19 ± 0.14ᵃ	27.11 ± 0.63ᵇ	17.68 ± 0.53ᶜ	25.75 ± 0.50ᵇ
Lymphocytes (%) **	49.61 ± 1.26ᵃ	66.31 ± 0.57ᵇ	54.79 ± 0.64ᶜ	60.43 ± 0.89ᵈ
Monocytes (%)***	1.30 ± 0.03ᵃ	3.12 ± 0.04ᵇ	2.10 ± 0.05ᶜ	2.09 ± 0.04ᶜ
Eosinophils (%)***	1.41 ± 0.02ᵃ	2.49 ± 0.03ᵇ	2.04 ± 0.02ᶜ	1.59 ± 0.04ᵈ
Hb (g/dl)***	13.48 ± 0.06ᵃ	8.65 ± 0.10ᵇ	12.53 ± 0.07ᶜ	9.12 ± 0.04ᵈ
HCT (%)***	55.93 ± 0.78ᵃ	44.19 ± 0.67ᵇ	39.10 ± 0.51ᶜ	50.36 ± 0.82ᵈ
MCV (fl)***	73.04 ± 0.36ᵃ	55.02 ± 0.69ᵇ	61.87 ± 0.89ᶜ	61.08 ± 1.29ᶜ
MCH (pg)***	19.84 ± 0.53ᵃ	12.41 ± 0.08ᵇ	16.79 ± 0.21ᶜ	16.28 ± 0.07ᶜ
MCHC (g/dl) ***	37.54 ± 1.12ᵃ	45.22 ± 0.68ᵇ	32.49 ± 0.65ᶜ	35.70 ± 0.78ᵃ
Platelet Count*** (×10³/µl)	653.88 ± 9.28ᵃ	445.00 ± 4.39ᵇ	617.10 ± 2.95ᶜ	462.93 ± 5.76ᵇ

[3] Red Blood Cells (RBC), Total Leukocyte Count (TLC), Hematocrit (HCT), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH),Mean Corpuscular Hemoglobin Concentration(MCHC), Platelet Count (PC) Microliter (µl), Femtoliters (fl), Picograms(pg), Deciliter (dl). Values are represented as Mean ± SEM (n=40) *** = Highly significant ($P < 0.001$), ** = Significant ($P < 0.01$), * = Moderately significant ($P < 0.05$) Groups sharing the same letter (a, b, c, d) are not significantly different ($P > 0.05$).

DISCUSSION

Bifenthrin, commonly used synthetic pyrethroid insecticide, is known to cause a range of toxic effects including developmental issues, neurobehavioral and immunotoxicity, oxidative stress, hormonal imbalances, and damage to vital organs particularly liver, lungs, kidneys, testes, and nervous system.[4] Despite being commonly present in the environment, studies focusing on the organ-specific toxicity of bifenthrin are still limited.

To address this research gap, this research was conducted to assess the toxic effects of bifenthrin on renal tissues in mice and to examine the ameliorative role of *Withania coagulans* (WCE) fruits extract against nephrotoxicity induced by bifenthrin. A range of toxicological parameters were analyzed, including behavioral alterations, histological and micrometric analysis, hematological profiles, and renal biomarkers.

No mortality was recorded in any group during the 10-day experimental period. However, mice exposed to bifenthrin displayed decreased locomotor activity due to neurotoxic effects of toxicant.[15] Regarding body weight, the control group showed no significant changes, whereas the BT group gained weight likely linked to bifenthrin's ability to facilitate fat deposition by increasing uptake of fatty acids and suppressing fat degradation as reported by.[16]

The Histopathological findings indicates that exposure to bifenthrin caused various significant micrometric and histopathological alterations including frequent atrophied glomeruli, intraglomerular capillaries congestion, increased periglomerular spaces, dilated proximal and distal tubules with loss of brush border, necrosis and vacuolization in collecting tubules, endothelial cells oedema, juxtamedullary haemorrhage, partial degradation of some cortical glomeruli and reduction in mean number of glomeruli per unit area. Ikram *et al*., 2021 also reported partial glomerular degradation, endothelial cell damage in bowman's capsule, intracellular oedema and swelling of glomeruli in response to bifenthrin exposure.[17] Similar results were reported by Tayyab *et al*., 2022 including swelling of PCT, intraglomerular capillaries congestion and renal tubular lesions in response to exposure to bifenthrin.[18] Rekha *et al*., 2013 also noticed similar changes of shrinkage of glomerulus and tubular dilation as a result of toxicant administration.[19] Similar results of glomerular atrophy, vacuolated tubules, wide bowman's space and lesions in renal tubules were noticed by Alshailabi *et al*., 2021 due to toxicant exposure.[20] Adeyele *et al*., 2024 also reported similar findings shrinkage of glomerulus, widened periglomeruli space and tubular necrosis due to administeration of toxicant.[21]

Mechanistically, these pathological changes are consistent with bifenthrin induced oxidative stress and inflammatory signaling. Previous studies have shown that pyrethroids increase the production of reactive oxygen species (ROS), lipid peroxidation, and mitochondrial dysfunction, which trigger necrosis in renal tissues and cell apoptosis .[17,21] Excess ROS also destabilizes membrane lipids and proteins, reducing

the efficiency of glomerular filtration and tubular reabsorption and thereby increasing serum creatinine and urea.

Bifenthrin has further been shown to activate the renin–angiotensin–aldosterone system (RAAS), increasing angiotensin II and vasopressin release, which results in renal ischemia, vasoconstriction, and altered glomerular hemodynamics.[5-6] Inflammation is another critical mediator, as bifenthrin elevates pro-inflammatory cytokines such as TNF-α, IL-2, and IL-6, contributing to interstitial infiltration and tubular necrosis.[7]

The increase in size of proximal and distal tubules might be in response to selective accumulation of nephrotoxic drug into these segments of nephron. The toxicant induced alterations in membrane integrity, ATP depletion and cytoskeleton component might be resulted in loss of brush border in proximal convoluted tubules.[20] All these stress conditions might have contributed to glomerular and renal tubular damage seen in the present study.

The bifenthrin and *Withania coagulans* combined treatment group (BWCT) exhibited milder pathological alterations as compared to group treated with bifenthrin. Histology was impaired including rehabilitation of structure of glomeruli, particularly partial recovery from glomerular atrophy, rehabilitation of proximal and distal renal tubules in terms of CSA of tubules, increase in glomeruli size and reduction of periglomerular space. Although, both groups showed some similarities such as alterations in glomerular and tubular structures, some signs of medullary hemorrhagia, the severity of these changes was notably milder in the WCE treated group. These results justify the ameliorative role of WCE by minimizing inflammation, vacuolization and necrosis.[3,13,22]

Several hematological changes were also observed in group treated with bifenthrin. Leukocyte counts rose sharply, indicating activation of certain components of immune defense system, while red blood cell indices (RBC, Hb, HCT, MCV, MCH) dropped significantly in BT group, resulting from destruction of erythrocytes in the blood of mice due to treatment with bifenthrin. Platelet counts dropped dramatically, in both BT and BWCT. In contrast, a significant increase in MCHC and percentage of neutrophils, lymphocytes, monocytes and eosinophils was observed. Similar results of reduction in Hb, RBC count, Total erythrocyte count and packed cell volume, while increase in TLC were reported by Tayyab *et al.,* 2022.[18] Adeyele *et al*., 2024 reported alteration in RBC count, hematocrit, Hb concentration and WBC count in response to bifenthrin exposure.[21] The findings of Ullah et al., 2022 showing reduced RBCs, Hb and PCV as well as increased WBCs and platelets supported these results, whereas their noted increase in MCV and MCH and drop in MCHC opposed these findings.[23] Significant decrease in RBC count and Hb concentration, hematocrit, while significant increase in WBC count and MCHC were in concurrence with the results of Kalsoom *et al*., 2024.[24]

These findings were in contrast with Dar *et al*., 2012 who reported no change in MCH and MCHC before and after treatment with bifenthrin.[25]

Withania coagulans extract showed a protective effect on hematological parameters by increasing RBC count, Hb levels, and reducing the abnormal rise in WBCs caused by bifenthrin exposure. It helped to restore parameters like HCT, MCV, MCH, and platelet count towards normal levels.

Renal function tests revealed elevated urea and creatinine levels in BT group, signaling significant hepatocellular impairment. Similar results were reported by Tayyab *et al*., 2022 including elevated levels of urea and creatinine in response to bifenthrin exposure.[18] Similar results of increase in serum urea and creatinine levels were reported by Adeyele *et al*., 2024.[21] Elevated levels of serum urea and creatinine indicate kidney damage as well as acute tubular necrosis resulting from the pesticide toxicity.[26] Kidney damage makes the kidney inefficient to eliminate both urea and creatinine, leading to their accumulation in the bloodstream. Studies indicate that the high serum rates of creatinine and urea in mice indicate necrosis of kidney epithelium, increased size of proximal tubules associated with interstitial inflammation, damage in the collecting tubules of nephron.[20,27] The results of renal function tests showed that serum creatinine and urea levels were significantly reduced in the *W.coagulans* treated group as compared to the bifenthrin treated group, indicating that WCE treatment reduced the levels of urea and creatinine demonstrating a protective effect of *W.coagulans* on kidney function.[3,22]

The histopathological and biochemical findings indicate that WCE plays a significant role in mitigating kidney damage caused by bifenthrin toxicity. *Withania coagulans* helps protect against bifenthrin toxicity by reducing the activity of apoptosis-related enzymes like caspases, limiting cell damage and stabilizing mitochondria.[3] These protective effects can be mechanistically linked to the phytochemical composition of *W. coagulans*, which is rich in withaferin A, withanolides, flavonoids and coagulin. These compounds exert strong antioxidant activity by neutralizing ROS, stabilizing mitochondrial membranes and enhancing superoxide dismutase (SOD) and catalase activity. WCE prevents glomerular collapse and tubular dilation by reducing lipid peroxidation and protecting membrane integrity observed under bifenthrin stress.[13] Furthermore, *W.coagulans* exhibits potent anti-inflammatory properties by suppressing the activation of NF-κB and downregulating proinflammatory cytokines (TNF-α, IL-6).

Our results with *Withania coagulans* were consistent with earlier studies where natural antioxidants such as propolis and pollen ameliorated renal injury by modulating oxidative and inflammatory stress.[28-29] Similarly, organoselenium compounds demonstrated protective effects on kidney tissues through their strong antioxidative properties.[30] These findings reinforced that the nephroprotective effects of *W.coagulans* were mediated through antioxidant and anti-inflammatory mechanisms, supporting its role as a natural therapeutic agent against renal toxicity.

This study was conducted with a relatively small cohort of 40 mice which may limit the generalization of findings. The 10 day exposure and treatment period might also not fully be able to assess the long term effects of bifenthrin toxicity or *W.coagulans* efficacy. Moreover, these were specie specific response which might differ for humans and other animals due to metabolic and physiological differences.

CONCLUSIONS

Withania coagulans (WCE) fruit extract have potential nephroprotective effects against bifenthrin induced nephrotoxicity in mice. Histological examination revealed that WCE treatment reduced the damage to kidney caused by bifenthrin exposure. Micrometric and biochemical studies further supported these findings, showing significant improvements in parameters in the WCE-treated group compared to the group exposed to bifenthrin. Future research could investigate the molecular mechanisms of WCE's protective effects and assess its potential to reduce bifenthrin toxicity in other organs.

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DISCLOSURE OF FINANCIAL AND NON-FINANCIAL RELATIONSHIPS AND ACTIVITIES AND CONFLICTS OF INTEREST

None

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