The Effect of *Bidens pilosa* Extract on Vancomycin-Induced Acute Kidney Injury in Prepubertal Rats

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Abstrak

Penggunaan Vancomisin dalam jangka panjang dan penggunaan dengan dosis yang tidak tepat masih memberikan efek samping yang tidak terkontrol secara fisiologi terutama dalam hal perkembangan dan metabolisme ginjal. Penelitian ini bertujuan untuk meneliti efek ekstrak *Bidens pilosa* (BPE) pada cedera ginjal akut yang diinduksi vancomicin pada tikus prepubertal. Metode penelitian ini menggukanan penelitian eksperimental dengan sebaran sebanyak 40 tikus betina wistar dibagi secara acak menjadi lima kelompok yang sama: control/Dewasa (C), Prepubertal (P), Prepubertal + *Bidens pilosa* (PB), Prepubertal+vancomicin(PV), dan Prepubertal+vancomicin+ *Bidens pilosa*. (PVB). Kerusakan ginjal dibuat dengan gentamisin untuk PG dan PVB. PB dan PVB diobati dengan dosis tunggal BPE. Darah, serum, dan jaringan ginjal diambil pada nekropsi untuk evaluasi. Hasil penelitian memperlihatkan jumlah total leukosit lebih tinggi di PB dibandingkan dengan yang lain (P=0,002). Peningkatan signifikan juga ditentukan pada kadar urea serum, kreatinin, aspartat aminotransferase, dan total protein pada PB, PV, dan PVB dibandingkan dengan C dan P. Tingkat glutation rendah dalam serum dan jaringan ginjal PV, dan tingkat malondialdehid tinggi dibandingkan dengan yang lain (P<0,05). Sebagai hasil dari penggunaan BPE pada cedera ginjal akut yang diinduksi vancomisin pada tikus ovariektomi, kadar kreatinin serum, urea, dan malondialdehid menurun pada PVB dibandingkan dengan PV, kadar glutation meningkat, dan tingkat keparahan temuan histopatologis menurun menjadi tingkat yang lebih ringan. Kesimpulan penelitian ini memperlihatkan dosis tunggal BPE sebagian mengurangi kerusakan ginjal pada tikus dengan cedera ginjal akut yang diinduksi gentamisin.

Kata kunci: cedera ginjal akut; vancomisin; tikus; *bidens pilosa* ; prepubertal; histopatologi.

Abstract

The long-term use of Vancomycin and its administration at inappropriate doses still cause uncontrolled physiological side effects, particularly in terms of kidney development and metabolism. This study aims to investigate the effects of Bidens pilosa extract (BPE) on vancomycin-induced acute kidney injury in prepubertal rats. This study employed an experimental research method, randomly dividing 40 female Wistar rats into five equal groups: control/adult (C), prepubertal (P), prepubertal + Bidens pilosa (PB), prepubertal + vancomycin (PV), and prepubertal + vancomycin + Bidens pilosa (PVB). Gentamicin caused kidney damage in both PG and PVB groups. We treated PB and PVB with a single dose of BPE. At necropsy, we collected blood, serum, and kidney tissue for evaluation. The research results showed that the total leukocyte count was higher in PB compared to the others (P = 0.002). Serum urea, creatinine, aspartate aminotransferase, and total protein levels significantly increased in PB, PV, and PVB compared to C and P. Glutathione levels were low in the serum and kidney tissue of PV, and malondialdehyde levels were high compared to the others (P<0.05). Using BPE in vancomycin-induced acute kidney injury in ovariectomized rats resulted in a decrease in serum creatinine, urea, and malondialdehyde levels in PVB compared to PV, an increase in glutathione levels, and a milder severity of histopathological findings. The conclusion of this study shows that a single dose of BPE partially reduces kidney damage in rats with gentamicin-induced acute kidney injury.

Keyword: accute kidney injury; vancomycin rats; bidens pilosa; prepubertal; histopathology

Introduction

Antibiotics fall into one of two categories: they either kill or stop the growth of germs. Bacterial death is how aminoglycosides function. There are several ways in which antibiotics harm bacteria. By preventing the synthesis of proteins, aminoglycosides affect microorganisms (1). Gram-negative bacteria can be effectively combatted by this class of narrow-spectrum antibiotics. These medications are helpful in treating a variety of illnesses, but they also have a number of negative side effects, such as teratogenic, neurotoxic, and ototoxic consequences (2). If taken for longer than five days, they can impair kidney function even at standard therapeutic dosages. Antibiotics belonging to the aminoglycoside category shouldn't be the main treatment option because of these adverse effects. They must be used carefully, particularly in people who have kidney impairment (3).

Anti-inflammatory medications, aminoglycoside antibiotics, and medications used to treat cancer can all have harmful effects on organs like the kidneys. Even when used at recommended therapeutic dosages, these drugs have the potential to seriously harm the kidneys. About 20% of patients have been documented to get acute renal failure as a result of therapeutic doses of vancomycin (4). Although the exact etiology of vancomycin-induced kidney injury is unknown, a number of possibilities have been put out. The detrimental impacts of free oxygen radicals may be a contributing factor, according to one notion backed by experimental research. In experimental research, rats have been given different doses and application techniques to cause kidney injury (5). Vancomycin is known to have quick effects at large dosages and can impair renal function even at regular dosages. Giving Vancomycin intraperitoneally for eight days at a dose of 100 mg/kg/day has been shown to cause nephrotoxicity.The foundation of homeopathy is the idea that like heals like, which states that while excessive dosages of a substance can exacerbate symptoms of an illness, low amounts of the same substance can cure it (6,7).

In veterinary medicine, supportive care is required for a number of illnesses. *Bidens pilosa* extract (BPE), a homeopathic remedy made from the B. pilosi spider and utilized as an adjuvant therapy, is one of those uses. This medication is mostly used in veterinary medicine to treat viral infections, traumatic necrotic disorders, edema, stimulate fast epithelialization, and treat some cancers (8). There have been reports of its efficacy in treating a variety of ailments in various animal breeds. BPE can be used on a variety of target species and is a reliable homeopathic medication in veterinary medicine, according to a number of early studies that are now accessible. It is used to treat purulent lesions, ulcers, and inflammatory illnesses because of its anti-inflammatory and regenerative qualities. Fatty acid peroxidation

produces glutathione in tissues. It has a very high intracellular concentration and is meant to guard against oxidative damage (9). It has been noted that the high glutathione levels in the proximal tubules make them more resistant to oxidative damage than the medulla. Oxidative stress is commonly measured using malondialdehyde (9).

Urine can assess this and blood can detect it. Since there are no particular markers for fatty acid oxidation, this correlates well with the degree of lipid peroxidation. The kidneys are influenced by sex hormones and other things. A number of renal illnesses are positively influenced by estrogen (10). Estrogen strengthens the immune system and has higher healing benefits. Furthermore, estrogen protects the kidneys. Sex hormones have been shown to significantly impact kidney damage. Rats' levels of progesterone, estrogen, and a number of other hormones will fluctuate throughout their cycle (11). In order to limit the effects of sex hormones and more precisely evaluate the effects of BPE on vancomycin-induced kidney injury, 19-day-old prepubertal rats that have not yet experienced an estrogen hormone cycle were employed. This study aims to determine the impact of BPE on vancomycin-induced kidney injury in young rats by assessing glutathione and malondialdehyde levels along with hematological and histological characteristics (12).

Methods

The Animal Ethics Committee of the Biology Study Program, Faculty of Mathematics and Natural Sciences, University of North Sumatra, has approved this research under the number: 0329/KEPH-FMIPA/2024. The study was carried out at the Department of Pharmacology, Faculty of Health Sciences, Universitas Haji Sumatera Sumatra. Rats Wistar rats were sourced from the Center for Application and Research of Experimental Animals at the University of North Sumatra, Medan. The daily intake of pellet feed and water by rats housed in standard cages was observed. In the study, all mice were maintained at a relative humidity of 40%-60%, an optimal room temperature of 22ºC, and a light/dark cycle of 12 hours each. This study utilized a control group comprising 40 female rats, with prepubertal rats weighing between 35-70 grams and adult rats weighing between 200-220 grams. At 19 days of age, the rats, obtained through self-breeding, were randomly assigned to five groups: control/Adult (C), prepubertal (P), *Bidens pilosa* (BP), Vancomycin (PV), and vancomycin + *Bidens pilosa* (PVB), with each group comprising eight rats. Prepubertal rats were sourced from a breeding process that lasted approximately 4 months prior to the initiation of treatment. This process included breeding, mating, and birthing, resulting in offspring that were 19 days

old and had not yet established a menstrual cycle, thus serving as the prepubertal samples for this study.

Group (C) comprises adult mice whose estrous cycles have been validated via cytological smear analysis. All mice in the other four groups were prepubertal, while the (P) group did not receive any additional treatment. The PB group was administered a single oral dose of PBE at 0.3 mg/kg. The PV group received vancomycin (15 mg/kg/IP) daily for one week to induce acute kidney injury. The PVB group received vancomycin at a dosage of 15 mg/kg via intraperitoneal injection for one week, along with a single subcutaneous dose of BPE at 0.3 mg/kg.

Blood Sample and Histology

At the conclusion of the study, all rats were euthanized via cervical dislocation while under general anesthesia(13). Blood samples were collected from the heart into serum tubes containing gel (BD Vacutainer®, BD, UK) and into tubes with K2EDTA (BD Vacutainer®, BD, UK). Blood samples designated for serum were maintained at room temperature for approximately one hour before being centrifuged at 3000 rpm for ten minutes with the Hettich Rotina 380R® (Hettich, Germany). The kidney tissue sample designated for biochemical analysis was homogenized in a phosphate buffer solution, after which the homogenate was extracted. All samples were maintained at -20ºC prior to analysis.

Biochemistry analysis and Hematology

Blood samples collected in K2EDTA were analyzed for total leukocyte count (WBC x103/µL) and additional hematological parameters utilizing a complete blood count device (VG-MS4e®, Melet Schloesing, France). Serum urea, creatinine, total protein (TP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were quantified utilizing a fully automated biochemical analyzer (Mindray BS120®, Mindray Medical Technology Istanbul, Turkey). The measurement of malondialdehyde in serum and tissue homogenate was performed following the established procedure, as was the measurement of glutathione. A microtiter plate spectrophotometer reader (Spectramax Plus®, Marshall Scientific, Product Code: MD-SMP, USA) was utilized to collect the data.

Histopathology procedure

Histopathological analysis was performed at the North Sumatra Veterinary Center. Kidney tissue was fixed in 10% buffered formalin for 72 hours, subsequently washed under running water for 15-20 minutes to eliminate formalin, and then dehydrated by sequential immersion in graded alcohol (75%, 96%, 100%). Following the cleaning of the tissue with xylene and subsequent infiltration with paraffin, the preparation of the paraffin block is conducted. Sections measuring 5 µm in thickness were stained using hematoxylin-eosin (Sigma-Aldrich®, Merck, Germany). Micrographs were obtained using a light microscope equipped with a DP21 camera system (Olympus BX43®, Japan) to assess morphological changes. The sections are evaluated according to histopathological findings categorized as none (-), mild (+), moderate (++), or severe (+++) (14).

Immunohistochemical Analysis(15)

After deparaffinization with xylene and rehydration with graded alcohols (100%, 96%, 75%) for two minutes, the sections are heated with 10mM citrate buffer for ten minutes in a microwave for antigen retrieval. The parts were coated with a hydrophobic pen and washed with a 0.1M sodium phosphate buffer solution. (PBS). To inhibit endogenous peroxidase activity, they were incubated with a 3% hydrogen peroxide solution for five minutes at room temperature (RT), washed with PBS, and incubated with a blocking solution for ten minutes at RT. After removing the blocking solution, the sections were incubated with primary antibodies anti-BAX (FNab000810, dilution 1:100) and anti-Caspase9 (FNab01295, dilution 1:100) at +4ºC overnight. The kidney sections were washed with PBS and incubated with secondary antibodies for ten minutes at room temperature.

Before incubating with horseradish peroxidase (HRP) polymer for ten minutes at room temperature, the blocks were washed with PBS. The sections were incubated with a 3,3-diaminobenzidine (DAB) solution for two minutes, and the DAP reaction was stopped with distilled water. Harris hematoxylin was used as a counterstain. The sections were washed with distilled water and dehydrated with graded alcohol (75%, 96%, 100%) for two minutes and mounted with Entellan®.A Thermo Scientific™ UltraVision™ Quanto Detection System HRP DAB kit (Thermo Scientific™ TL-060- QHD) is used for hydrogen peroxide, blocking solution, secondary antibodies, and HRP polymer. For immunohistochemical analysis, five different areas of each slide are photographed at 20× magnification. The H-SCORE level is calculated using the formula H-SCORE=Σ Pi (i+1) [micrograph staining density (i), pixel ratio (Pi)]. Statistical Data Analysis is provided as mean ± standard error of the mean. (SEM).

Those groups do not conform to a normal distribution according to the histogram, Q-Q plot method, and Shapiro-Wilk test. The Kruskal-Wallis H test is used for multiple group comparisons, and the Mann-Whitney U test is used for pairwise comparisons. The adjusted P value was calculated by applying the Bonferroni correction to the P value obtained after the Mann-Whitney U test. The SPSS program (SPSS Version 23.0®, Chicago, IL, USA) was used for all statistical analyses. Differences between groups in terms of the parameters examined are considered significant at the $P<0.05$ level.

Data Analysis

Data are presented as the mean ± standard error of the mean (SEM). The groups exhibit non-normal distribution as indicated by the histogram, Q-Q plot, and Shapiro-Wilk test results. The Kruskal-Wallis H test facilitates multiple group comparisons, while the Mann-Whitney U test is employed for paired comparisons. The adjusted P value was determined by applying the Bonferroni correction to the P value derived from the Mann-Whitney U test. Statistical analyses were conducted using SPSS software (Version 23.0®, Chicago, IL, USA). The difference between groups regarding the examined parameters is deemed significant at the $P<0.05$ level.

RESULTS

The total leukocyte count in the PB group was significantly higher than in the other groups ($P=0.002$). Statistical significance was observed between groups for lymphocytes, granulocytes, monocytes, erythrocytes, hematocrit percentage, hemoglobin, and platelet count ($P<0.05$). Table 1. Significant increases in serum urea, creatinine, aspartate aminotransferase, and total protein levels were observed in the PB, PV, and PVB groups when compared to the C and P groups ($P<0.05$). Table 2. The PV group exhibited the lowest levels of serum and kidney tissue glutathione, alongside the highest levels of malondialdehyde ($P<0.05$). Table 2. Histopathological evaluation utilizing hematoxylin-eosin staining was performed to examine morphological alterations in kidney tissue resulting from BPH in rats experiencing vancomycin-induced nephrotoxicity. Figure 1. The kidney tissue in group C exhibits a normal appearance, while group P shows similarities to group C. The glomerulus and Bowman space are normal in group PB; however, mild glomerular segmentation is observed, in contrast to group P. Proximal tubule cells in the PV group exhibited swelling and cytoplasmic vacuolation, with certain regions displaying cytoplasmic degeneration. In comparison to the C

and P groups, there was observed dilation in the Bowman space, glomerular atrophy, and notable segmentation. The PV group exhibited numerous cells within the tubular lumen, suggesting acute tubular necrosis. The increase in hyaline casts within the proximal tubule lumen is notable.

Table 1. Hematological data in the study according to groups

Parameters	Groups (Mean±SEM)					P Value
	C	P	PB	PV	PVB	
Total leukocytes count (x10³/µL)	5.22±0.31[a]	4.22±0.71[a]	10.11±1.16[b]	5.27±0.74[ab]	6.88±0.53[ab]	0.003
Lymphocytes count (x10³/µL)	5.10±0.31	4.20±0.63	7.18±1.06	3.19±0.70	4.47±0.65	>0.05
Monocytes count (x10³/µL)	0.33±0.02[a]	0.27±0.04[a]	0.56±0.09[b]	0.42±0.05[b]	0.39±0.05[b]	<0.001
Granulocytes count (x10³/µL)	0.49±0.04[a]	0.21±0.13[ac]	1.83±0.06[b]	1.49±0.06[bc]	1.22±0.22[bc]	<0.001
Red blood cell count (x10⁶/µL)	8.21±0.18[a]	8.29±0.24[a]	13.58±2.34[b]	8.17±0.11[a]	7.52±0.13[a]	0.038
Mean red cell volume (fL)	80.91±0.66[a]	81.55±0.92[a]	76.60±1.25[ab]	74.21±0.55[b]	72.60±1.21[b]	<0.001
Hematocrit (%)	60.361 .24[a]	62.03±1.49[a]	80.85±8.78[b]	55.94±0.88[a]	56.86±0.90[a]	0.004
Mean erythrocyte hemoglobin (pg)	33.04±0.30[a]	33.93±0.38[ab]	29.38±2.03[a]	34.69±0.18[b]	33.41±0.20[ab]	0.011
Mean hemoglobin volume (g/dL)	42.51±0.16[a]	43.53±0.67[a]	41.88±2.31[a]	48.51±0.11[b]	47.55±0.67[b]	<0.001
Erythrocyte distribution width (fL)	20.45±0.44[ab]	21.73±0.17[a]	19.68±0.25[b]	19.89±0.17[b]	19.55±0.26[b]	0.001
Hemoglobin (g/dL)	26.40±0.40[a]	27.43±0.37[a]	31.30±1.48[b]	27.71±0.36[a]	27.59±0.24[a]	0.001
Platelet count (x10³/µL)	1059.88±27.29	2278.75±483.80	4487.00±1378.70	882.13±76.90	1107.38±114.47	>0.05
Mean platelet volume (fL)	15.16±0.04	15.23±0.13	15.30±0.06	15.21±0.05	15.15±0.04	>0.05
Platelets (%)	1.54±0.02[ab]	1.14±0.23[ab]	2.42±0.75[a]	0.45±0.04[b]	0.56±0.06[ab]	0.017
Platelet distribution width (fL)	17.20±0.17[a]	17.23±0.23[a]	17.95±0.23[ab]	18.55±0.23[b]	18.21±0.09[b]	0.007

***C**: Control group, **P**:Prepubertal, **PB**: Bidens pilosa group, **PV**:Vancomicin group, **PVB**:vancomycin +Bidens pilosa group*

[a-c] The mean values with different letters in the same line represent the difference between groups. P<0.05: Expresses statistical significance

Parameters	Groups (Mean±SEM)					P Value
	C	P	PB	PV	PVB	
Alanine aminotransferase (U/L)	61.11±4.02	57.13±3.33	60.51±5.53	72.03±5.80	64.74±2.40	>0.05
Aspartate aminotransferase (U/L)	144.62±8.54^{a}	147.03±6.81^{a}	151.49±5.37^{a}	187.84±15.64^{b}	205.51±6.73^{b}	<0.001
Urea (mg/dL)	66.57±5.98^{a}	67.36±7.06^{a}	78.74±3.23^{ab}	104.38±16.02^{b}	89.56±7.62^{ab}	0.017
Creatinine (mg/dL)	0.76±0.11^{a}	0.94±0.09^{a}	0.83±0.06^{a}	2.73±0.08^{b}	2.45±0.23^{b}	<0.001
Total protein (g/dL)	7.26±0.19^{a}	7.25±0.13^{ab}	7.80±0.15^{bc}	8.18±0.19^{c}	7.83±0.11^{bc}	<0.001
Glutathione (umol/mL)	1.42±0.11^{ab}	1.31±0.09^{ab}	1.52±0.12^{b}	1.09±0.05^{a}	1.12±0.06^{a}	0.007
Malondialdehyde (nmol/mL)	1.43±0.06^{a}	1.46±0.05^{a}	1.51±0.14^{a}	2.15±0.11^{b}	2.02±0.12^{b}	<0.001
Kidney tissue glutathione (umol/g)	7.90±0.12^{a}	8.15±0.26^{a}	9.33±0.50^{a}	7.62±0.25^{a}	11.89±0.81^{b}	<0.001
Kidney tissue malondialdehyde (nmol/g)	9,51±0,34^{a}	9,69±0,49^{a}	10,58±0,40^{ab}	13,51±0,56^{c}	12,25±0,45^{bc}	<0.001

__C__: Control group, __P__:Prepubertal, __PB__: Bidens pilosa group, __PV__:Vancomicin group, __PVB__:vancomycin +Bidens pilosa group. __SEM__: standard error of mean. $^{a-c}$ The mean values with different letters in the same line represent the difference between groups. P<0.05: Expresses statistical significance

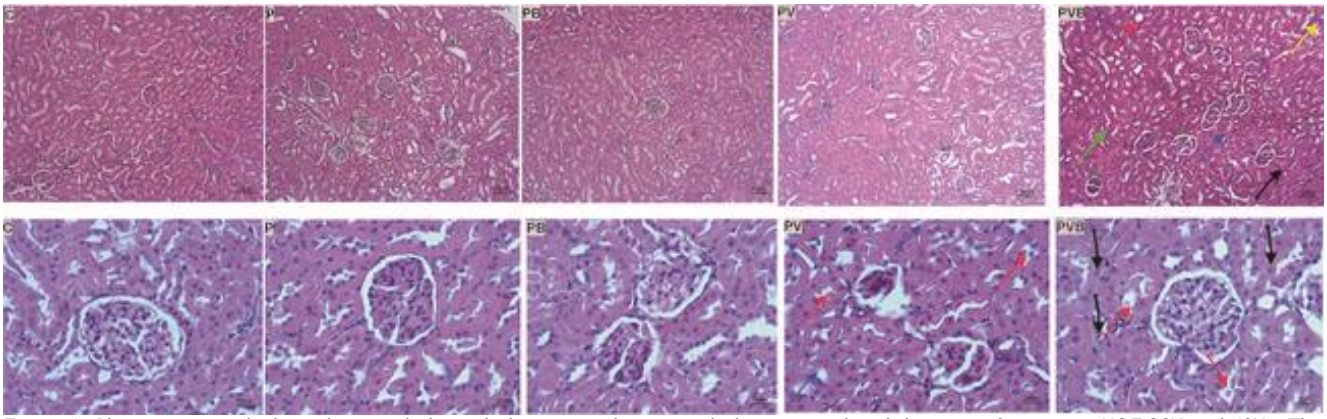

Figure 1. Photomicrograph shows histopathological changes in the mouse kidney stained with hematoxylin-eosin. (H&E 20X and 40X). The kidney tissue of group C and group O has a normal appearance, while group OT has mild glomerular segmentation. (GS). In the OG and OGT groups, cytoplasmic vacuoles (black arrows), glomerular atrophy (GA), Bowman space dilation (stars), hyaline cylinders (red arrows) in the tubular lumen, cytoplasmic degeneration (blue arrows), cells in the proximal tubular lumen (yellow arrows), and enlarged cells (green arrows) were observed; although there were variations in their density. Abbreviations; G: Glomerulus, DT: Distal tubule, PT: Proximal tubule, C: Control group, P: Prepubertal group, PB: *Bidens pilosa* group, PV: Vancomycin group, PVB: Vancomycin+Tarantula cubensis group. Bar (top row): H&E X10, Bar (bottom row): H&E X40

Table 3. Skor kerusakan histologis pada jaringan ginjal

Parameters	C	P	PV	PV	PVB
Dilatation in Bowman space	-	-	+++	-	++
Glomerular segmenting	-	-	+++	+	+++
Cells in tubules lumen	-	-	++	-	++
Cytoplasmic vacuole in tubules cells	-	-	+++	+	++
Hyaline cylinders in tubules lumen	-	-	++	-	+

Sections were evaluated according to histopathological findings as none (-), mild (+), moderate (++) and severe (+++). __C__: Control group, __P__: Prepubertal group, __PB__: Bidens pilosa group, __PV__: Vancomicin group, __PVB__: Vancomicin + Bidens pilosa group

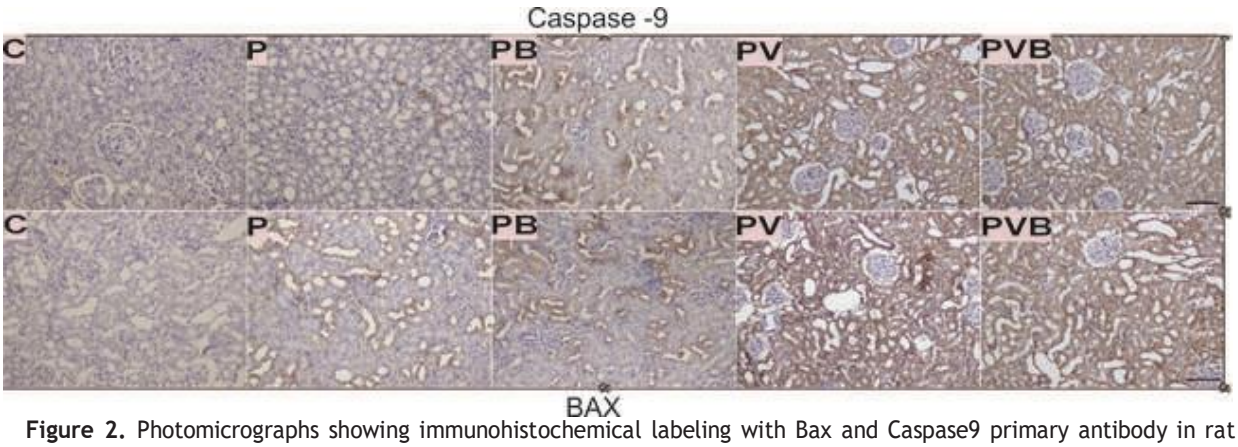

Figure 2. Photomicrographs showing immunohistochemical labeling with Bax and Caspase9 primary antibody in rat kidney (20X magnification). Brown staining indicates positive immune-reactivity. Caspase-9 and Bax proteins are secreted when cells are prone to apoptosis. Positive immune-reactivity areas indicate the susceptibility of cells to apoptosis. Compared to the other groups, the PV and PVB groups have more apoptotic cells rates because of positive immune-reactivity. Abbreviations; C: Control group, P: Prepubertal group, PB: *Bidens pilosa* group, PV: Vancomycin group, PVB: Vancomycin+*Tarantula cubensis* group

Certain regions exhibit cytoplasmic degeneration. Dilation in the Bowman space, glomerular atrophy, and prominent segmentation were observed in comparison to groups C and P. The PV group exhibited numerous cells within the tubular lumen, suggesting acute tubular necrosis. The increase in hyaline casts within the proximal tubular lumen was notable. The comparison of the PVB group and the PV group revealed dilation in the Bowman space, the presence of cells in the tubular lumen indicative of acute tubular necrosis, and a reduction in the proportion of hyaline casts within the proximal tubular lumen. The segmentation of the glomerulus occurs at an equivalent level. Table 3 presents the histological damage scores in kidney tissue. Apoptosis was induced due to nephrotoxicity from vancomycin in the PV and PVB groups (Fig. 2). The H-score for BAX and caspase-9 by group demonstrated statistical significance ($P<0.05$) Figures 3.

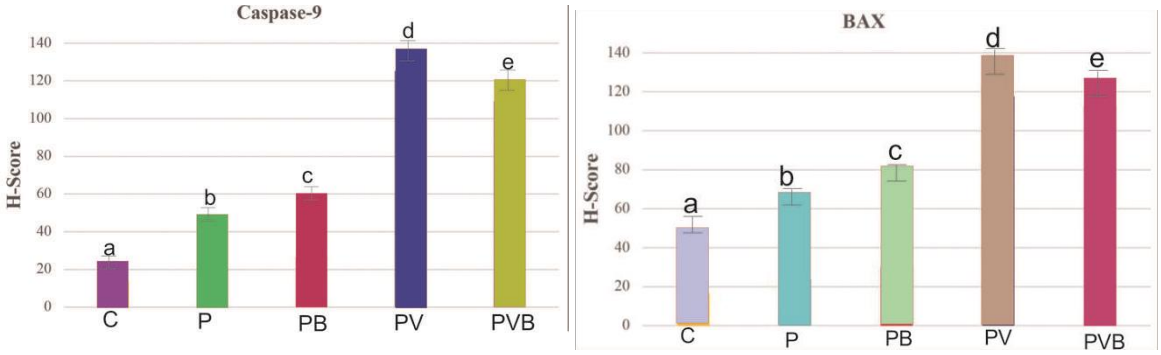

Fig3. H-scoreofCaspase-9 bygroups.**C:**Controlgroup, **P:** Prepubertal group, **PB:** *Bidens pilosa* group, PV: vancomycin group, **PVB:** vancomycin + *Bidens pilosa* group. $^{a-c}$ Different letters represent the difference between groups. P<0.05: Expresses statistical significance **(Left: Caspase 9 and Right: BAX)**.

Alterations in hematological and serum biochemical parameters offer critical insights to clinicians concerning disease severity, treatment efficacy, metabolic processes, and organ functionality. A study on rats demonstrated comparable findings regarding hematological parameters with our PV group, particularly concerning WBC levels. The elevated WBC

levels in the PB group, in comparison to other groups, are attributed to the regenerative properties of BPE within the tissue(16,17). High doses of vancomycin induce nephrotoxicity by disrupting the cell membrane, allowing the drug to enter the cytoplasm. Vancomycin in the cytosol activates the intrinsic apoptosis pathway through its impact on mitochondria, leading to an increase in Bax expression. Bax subsequently elevates the synthesis of cytochrome-c, thereby promoting apoptosis. Previous studies have demonstrated that cytochrome-c activates the caspase pathway. Recent research indicates that vancomycin quantifies oxidative stress. The findings indicate that elevated oxidative stress correlates with reduced glutathione levels and increased malondialdehyde levels in the PV group, aligning with existing literature(18). The histopathological alterations observed in the kidney tissue of rats subjected to vancomycin-induced nephrotoxicity align with prior research findings. Consistent with the literature, the PV group exhibited glomerular atrophy, hyaline casts in the tubular lumen, tubular vacuolization, tubular cell desquamation, and glomerular segmentation(19). The introduction of BPE resulted in a reduction of Bowman's space dilation, cytoplasmic vacuoles in tubular cells, and the proportion of hyaline casts within the tubular lumen(18,20). Cell desquamation and the rate of glomerular segmentation in the tubular lumen remain constant. The histopathological comparison between the PVB and PV groups revealed only minor differences, likely attributable to the administration of a single dose of BPE. Our results indicate that a single dose of BPE offers partial protective and therapeutic effects(21). The protective and therapeutic effects of BPE are expected to enhance with repeated administration. Further research is required to validate this assumption. Induce apoptosis due to nephrotoxicity, aligning with existing literature. Nephrotoxicity is a significant side effect associated with vancomycin. An increase in serum creatinine signifies nephrotoxicity, typically associated with the dosage and duration of administration(22). A study on rats revealed elevated serum urea and creatinine levels in the vancomycin group relative to the control group. The comparable outcomes in the PV and PVB groups in this study suggest the occurrence of nephrotoxicity(23). A separate study found that BPE exhibits a protective effect against nephrotoxicity and reduces concentrations of urea and creatinine. The current study aligns with existing literature, demonstrating that serum creatinine and urea concentrations are lower in the PVB and PV groups, suggesting that BPE partially mitigates acute kidney injury(24).

Conclusions and Suggestions

In the conclusions we argue that these results are due to the antiphlogistic and regenerative properties of BPE, as well as its healing effects on necrotic tissue. A study conducted on rats reported that the levels of malondialdehyde in the vancomycin group were higher compared to the control and BPE groups. Glutathione in tissues aims to protect against oxidative damage, and malondialdehyde is widely used for confirmation.

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