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Protective effects of Periwinkle (Catharanthus roseus) extract on Renal Caspase-3 and Interleukin-6 expression in cadmium-exposed mice

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Abstract: Cadmium is a toxic heavy metal known to induce oxidative stress, DNA damage, and inflammation, particularly in kidney tissue, as shown by elevated levels of inflammatory cytokines such as Interleukin-6 (IL-6) and apoptotic markers like Caspase-3. This study aimed to evaluate the protective effect of periwinkle (*Catharanthus roseus*) extract against cadmium induced kidney damage in male Balb/C mice. Twenty mice were randomly divided into four groups: a healthy control group (KS) receiving only pellets and water; a negative control group (KN) receiving intraperitoneal cadmium chloride at 4.5 mg/kg body weight/day; a positive control group (KP) treated with cadmium and vitamin E (50 IU/kg BW/day); and a treatment group (P1) given cadmium along with oral periwinkle extract at a dose of 500 mg/kg BW/day for five days. Kidney tissues were collected post-treatment, by six days, levels of IL-6 and Caspase-3 were measured using ELISA. The results showed that the periwinkle treated group had significantly lower IL-6 levels (59.4±17.9) compared to the cadmium-only group (115.1±5.85), and lower Caspase-3 levels (247.1±4.9) compared to the negative control (271.3±14.2), with statistical significance ($p<0.05$). These findings suggest that periwinkle extract exerts a protective effect by reducing inflammation and apoptosis in kidneys exposed to cadmium toxicity.

Keywords: Periwinkle; Caspase-3; Interleukin-6; cadmium.

INTRODUCTION

Cadmium is a type of heavy metal that has a high toxicity value after mercury (Hg). The maximum levels in wastewater allowed based on the Decree of the State Minister of Environment Number: KEP-51/MENLH/10/1995 concerning Liquid Waste Quality Standards for Industrial Activities is 0.05 ppm. Cadmium is included in the category of non-essential heavy metals, namely metals whose still unknown biological function or is potentially toxic in the body, therefore, its presence must be carefully monitored, as elevated levels can potantially severely impact health, especially in waters.[1] Cadmium is used as the main material or additive material in Various industry, including the electroplating industry, nickel-cadmium batteries, coating materials, stabilizer materials in the plastic industry and other synthetic goods.with heavy used of it in industries there is always potantial to harm health through the food chain beside that there also high chance Animals will easily absorb cadmium from food and accumulate in tissues such as kidneys, liver, and reproductive organs.[2]

Cadmium poisoning is reported to occur in Japan which results in lumbago disease which can cause bone damage, other organ like The kidneys are also a

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primary target organ, when the content reaches 200 µg Cd/gram (wet weight) in the renal *cortex it can* resulting in kidney failure and even death. The population most at risk is includes *postmenopausal* women who are malnourished, deficiedncies in vitamin D and calcium . In addition, cadmium accumulation in the body increases significantly between at the age of 20-30 years.[3]

The accumulation of cadmium in the body contributes to oxidative stress resulting in cellular DNA damage and inflammation, triggering inflammation characterized by an increase in inflammatory cytokines, including Interleukin-6 and leading to cell apoptosis.[4] once inside the body, it binds to metalotionin to form Cd+Mt bonds, then it will be deposited in the kidney organs and induce the formation of free radicals, resulting in lipid peroxidation which can damage the structure of the kidney organs.[5]

Kidney cell damage is characterized by an increase in the expression of Caspase 3, often associated with decrease kidney function which is marked by the presence of proteinurea in the urine.[6] previous studies, stated that plants such as periwinkle (*Catharantus roseus*) possess high antioxidants properties, another studes conducted to test DPPH on different concentrations (200, 400, 600, 800, 1,000 µg), shown of the five concentrations tested 800µg had the highest antioxidant activity.[7]

Several natural compounds have been previously investigated for their protective effects against cadmium-induced nephrotoxicity, including antioxidants such as vitamin C, curcumin, and resveratrol, which demonstrated significant anti-inflammatory and anti-apoptotic properties. Among these, vitamin E has been extensively investigated, with recent studies showing that it significantly ameliorates cadmium-induced renal oxidative stress and apoptosis in animal models[8,9]. While vitamin E has been widely studied, studies evaluating the protective role of Catharanthus roseus remain very limited, However, the specific effect of periwinkle extract on IL-6 and Caspase-3 levels in cadmium-induced renal injury has not yet been studied. thus justifying the need for further research

Therefore, this study aims to evaluate the protective effect of Catharanthus roseus extract on renal Caspase-3 and Interleukin-6 levels in male Balb/C mice exposed to cadmium.

MATERIAL AND METHOD

Research materials include cadmium, water for injection, periwinkle extracts, aquades, ethanol, rat feed, and chloroform. This study is an experimental animal study with a complete randomized design with five replicates of each treatment. The study subjects used 1-2 month old male BalB/C mice with a body weight of 20-30 grams that were declared healthy and suitable for use for research by veterinarians, adapted for 7 days. The research subjects were divided into 4 groups, namely the healthy group consisting of 5 mice (KS), the negative control group (KN) consisting of 5 mice who received cadmium induction of 4.5 mg/kgBB/day by intraperitoneal injection (IP) for 5 days, the positive control group (KP) who received cadmium induction and vitamin E at a dose of 50 IU orally for 5 days, and the treatment group (P1) consisted of 5 test animals who received intraperitoneal cadmium at a dose of 4.5 mg/kgBB/day and periwinkle extract 500 mg/kgBB/day for 5 days.

Treatment protocol

The production of periwinkle extract and treatment was carried out at the Integrated Technical Implementation Unit of the SCCR Laboratory, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, This experiment was conducted in February 2024 at the SCCR Laboratory under approval from the Health Research Ethics Commission of the Faculty of Medicine, Sultan Agung Islamic University, with ethical clearance number 82/II/2024/Komisi Bioetik

Approximately 600 grams of periwinkle were chopped, dehydrated at a temperature of 50–60°C, and ground into dry powder. The powder was extracted through a maceration process using 95% ethanol, followed by filtration, and the residue was re-macerated using the same method. Ethanol was removed using a rotary evaporator to obtain a thick extract, which was then stored at 2–8°C until use.

The dosage of periwinkle extract was determined based on previous studies, which reported a protective effect on renal function at a dose of 500 mg/kg body weight per day. This dose was therefore selected for the treatment group. All mice were acclimatized for one week prior to treatment. under standardized environmental conditions (temperature 22±2°C, humidity 50–60%, and a 12-hour light/dark cycle) with ad libitum access to food and water Cadmium chloride was administered intraperitoneally at a dose of 4.5 mg/kg body weight per day, dissolved in water for injection, and given to the negative control, positive control, and treatment groups. The treatment group (P1) received periwinkle extract orally at 500 mg/kg body weight per day via gavage alongside cadmium induction. The positive control group (KP) received vitamin E orally at 50 IU/kg body weight per day with cadmium, while the negative control group received cadmium only. The healthy control group (KS) received standard feed and water without any treatment.

The mice after 24 hours after the last treatment were killed by means of cervical dislocation for the process of harvesting kidney organs. Kidney samples were taken and homogenized using RIPA buffer then centrifuged for 10 min at a rate of 1000 g at 4°C. Supernatants are taken and stored at a temperature of -200 C until the analysis process. The levels of IL-6 and Caspase-3 were measured using ELISA kits.

Analysis of Interleukin-6 and Caspase-3 levels using ELISA

Examination the levels of IL-6 and Caspase 3 using ELISA The levels of IL-6 and Caspase-3 were measured using commercially available rat ELISA kits according to the manufacturer's protocols. For IL-6, the Rat IL-6 ELISA Kit (Elabscience, Cat. No. E-EL-R0015) used with a detection range of 12.5–800 pg/mL. For Caspase-3, we used the Rat Caspase-3 ELISA Kit (ELK, Cat. No. ELK1528) with a detection range of 0.16–10 ng/mL. All assays were performed with four technical replicates (quadruple) to ensure accuracy and reproducibility. Standardization Preparation is as follows,: Preparation of standards containing known levels of IL-6 and Caspase 3 to create a standard curve. Blocking: A step to prevent nonspecific bonding by closing the remaining empty area on the microtiter plate. Sampling and Control: Addition of samples (serum, plasma, or cell supernates) to be tested, as well as positive and negative controls. Incubation: Incubate the plate so that IL-6 and Caspase 3 from the sample and standard are captured by the antibodies present in the plate. Washing: Cleaning the microtiter plate from unbound substances. Administering Detection Antibodies: Adding specific detection antibodies against IL-6 and Caspase 3 that bind to the target. Addition of Conjugate Enzymes: Adding enzymes bound to detection antibodies. Substrate Feeding: Addition of an enzymatic substrate that will produce a colored signal. Reaction Stop and Reading: The reaction is,Data then analys normality and homogeneity of variances were evaluated using the Shapiro–Wilk and Levene's tests, respectively. When both assumptions were satisfied, a one-way ANOVA was performed across groups, followed by Fisher's Least Significant Difference (LSD) post-hoc.

RESULTS AND DISCUSSION

Phytokimia profile of Periwinkle

The samples used in this study used Periwinkle plants (*Catharanthus roseus*) which were grown and extracted in the SEMARANG SCCR laboratory. Periwinkle extract is proven to contain alkaloid compounds, saponins, tannins, flavonoids, steroids and glycosides. In this study, the activity of periwinkle extract will be evaluated on the level of caspase-3 and interleukin-6 levels of kidneys in cadmium poisoning.

Table 1. Data from the study of caspase-3 and interleukin-6 levels

Variable	Group				p value
	Healthy (KS) n=5 mean±SD	Negative control (KN) n=5 mean±SD	Positive control (KP) n=5 mean±SD	Treatment n=5 (P1) mean±SD	**P<0.05**
Interleukin-6 Level	46.8±2.88	115.1±5.85	43.0±3.27	59.4±17.9	
Saphiro wilk	0.207	0.500	0.451	0.421	
Levene test					
One way Anova					0.000
Up to Caspase-3	37.6±9.57	271.3±14.2	249.8±24.4	247.1±4.9	
Saphiro wilk	0.413	0.136	0.065	0.156	
Levene test					
One way Anova					0.000

Effect of Periwinkle on Interleukin-6 Levels

Interleuikin-6 levels are a type of pro-inflammatory cytokine that is secreted and secreted by immune cells including monocytes, macrophages and T cells. In this study, the results of Interleuikin-6 levels in the treatment group were lower (P1= 59.4±17.9) than in the negative control group (KN =115.1±5.85), but the levels of Interleuikin-6 in the positive control group (KP = 43.0±3.27) had a lower trend than in the treatment group (Figure 1).

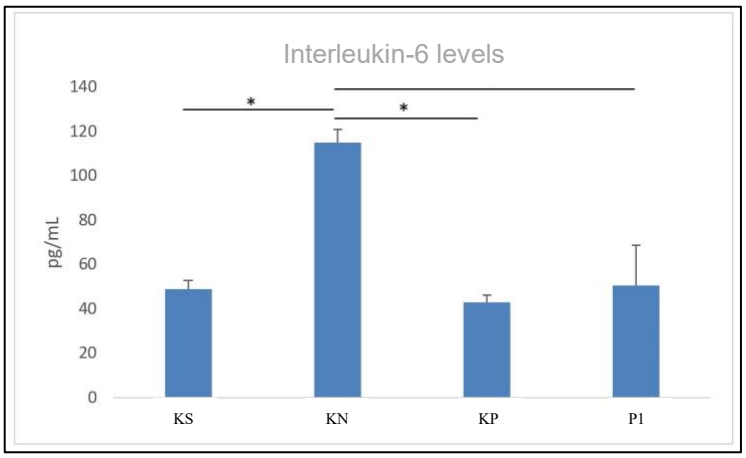

Figure 1. The results of the ELISA test showed the ratio of Interleukin-6 levels in the kidney tissue of each treatment group. *Shows a significant difference between the control group and the p<0.05 treatment

Table 2. *Post-hoc* LSD test of Interleukin-6 levels between treatment groups

Group	Group comparison	Significance
Healthy	Negative control	0.000
	Positive control	0.538
	Treatment	0.058
Negative control	Positive control	0.000
	Treatment	0.000
Positive control	Treatment	0.017

The results of *the post-hoc LSD test* are presented in the form of graphs in figure 1 and table 2 shown normality and homogeneity of variance were confirmed using the Shapiro-Wilk and Levene's tests, respectively ($p > 0.05$ in all groups), indicating that the data met the assumptions for parametric analysis. *One-way ANOVA* was performed and revealed a statistically significant difference in IL-6 levels across the four groups ($p = 0.000$), suggesting that the treatments had a measurable effect on the inflammatory response.

To further explore these differences, a *post-hoc LSD* test was conducted. The IL-6 level in the negative control group was significantly higher than in all other groups, including the treatment group ($p = 0.000$). The treatment group also showed a statistically significant reduction compared to the negative control, although it remained significantly higher than the positive control ($p = 0.017$). There was no significant difference between the treatment and healthy groups ($p = 0.058$), suggesting near normalization of IL-6 levels with periwinkle extract, although not to the extent achieved with vitamin E.

Effect of periwinkle extract on caspase level 3

Caspase 3 is a family of cysteine proteases that serve as the main effector during apoptosis for the proteolytic disassembly of most cellular structures, including the cytoskeleton, cell junctions, mitochondria, endoplasmic reticulum, Golgi, and nucleus. Apoptosis is a form of programmed cell death that removes individual cells in an organism in maintaining the overall structure of the surrounding tissues.

In this study, the results of the caspase-3 level test in the treatment group were lower (KP=247.1±4.9) than the negative control (KN=271.3±0.23), but the caspase-3 level in the positive control group (KP=249.8±24.4) did not have a significant difference compared to the treatment group (Figure 2).

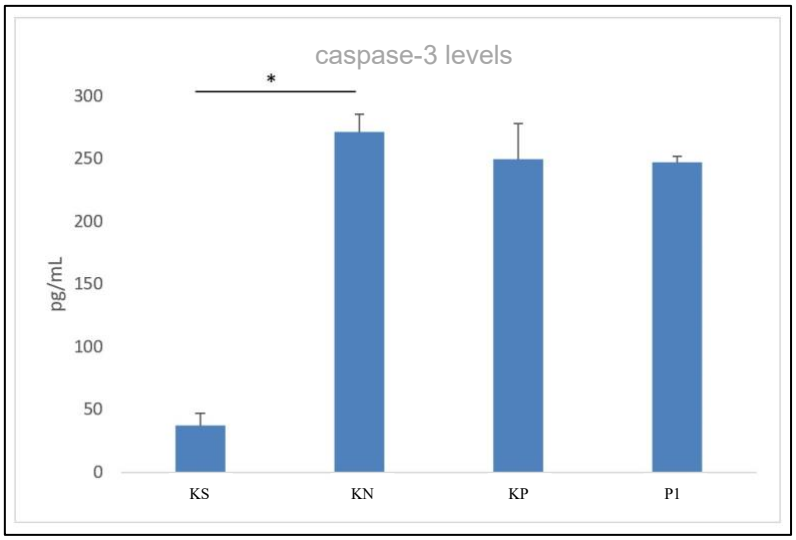

Figure 2. The results of the ELISA test showed the ratio of caspase-3 levels in the kidney tissue of each treatment group. *Shows a significant difference between the negative control group and the p<0.05 treatment and shows no significant difference between the control group and the p>0.05 treatment

Table 3. *Post-hoc* LSD test of caspase-3 levels between treatment groups

Group	Group comparison	Significance
Healthy	Negative control	0.000
	Positive control	0.000
	Treatment	0.000
Negative control	Positive control	0.039
	Treatment	0.017
Positive control	Treatment	0.785

Based The results of *the post-hoc LSD* test are presented in figure 2 and table 3, it can be seen that the administration of 500mg/kgBB dosage treatment provides a significant difference in reducing the level of caspase 3 compared to the negative control, but in the treatment group compared to the positive control group there is no significant difference. This showed The Shapiro-Wilk and Levene's tests confirmed that the Caspase-3 data were normally distributed and had equal variances across groups ($p > 0.05$), justifying the use of *one-way ANOVA*. The *ANOVA* test showed a significant overall difference among the four groups ($p = 0.000$).

Post-hoc LSD analysis revealed that the treatment group had significantly lower Caspase-3 levels than the negative control ($p = 0.017$), indicating reduced apoptosis. There was no statistically significant difference between the treatment and positive control groups ($p = 0.785$), suggesting that periwinkle extract was equally effective as vitamin E in mitigating cadmium-induced apoptosis. However, both the treatment and positive control groups remained significantly higher than the healthy group ($p = 0.000$), which indicates that while both treatments reduced cell death, they did not fully restore normal levels.

This study evaluated the effect of *Catharanthus roseus* (periwinkle) extract on renal inflammation and apoptosis in a mouse model of cadmium-induced nephrotoxicity. The results demonstrated that periwinkle extract significantly reduced Interleukin-6 (IL-6) and Caspase-3 levels in kidney tissue compared to the cadmium-only group. These findings indicate the extract's potential anti-inflammatory and anti-apoptotic effects, consistent with the hypothesis that *C. roseus* provides protection against heavy metal-induced kidney damage.

Cadmium is a highly toxic heavy metal that poses serious environmental and health risks. It is not essential to the human body and, once absorbed, tends to accumulate in vital organs, especially the kidneys.[1,10] Prolonged exposure leads to oxidative stress and inflammation through the activation of intracellular pathways such as NF-κB, which in turn upregulates pro-inflammatory cytokines like IL-6.10 Studies have shown that cadmium accumulation is a key contributor to chronic kidney injury, and its renal toxicity has been widely demonstrated in both animal and human studies.[11]

Cadmium exposure not only activates NF-κB but also disrupts mitochondrial homeostasis, leading to increased production of reactive oxygen species (ROS) and mitochondrial swelling precursors of apoptosis.[12] Inflammatory signaling further contributes to fibrosis and reduced glomerular filtration rate, as seen in long-term exposures.[13,14]

Experiment shown mice who exposed to cadmium showed elevated IL-6 and Caspase-3 levels, confirming cadmium's role in promoting inflammation and apoptosis. These results are consistent with previous findings, where cadmium increased pro-inflammatory cytokines and triggered apoptotic pathways in kidney tissue.[15,16] The role of IL-6 as a central inflammatory mediator is well established in renal pathology. It acts through the JAK/STAT and PI3K pathways, amplifying the inflammatory response and promoting cell death.[17,18]

The protective effects observed in the periwinkle-treated group likely stem from its bioactive constituents. *C. roseus* is rich in flavonoids, tannins, and alkaloids such as vinblastine and vincristine, which contribute to its antioxidative and anti-inflammatory properties.[19] Flavonoids such as quercetin and kaempferol have been reported to downregulate NF-κB activation and enhance the expression of Nrf2, thereby reducing oxidative stress and cytokine production.[20,21] This mechanism could explain the significant decrease in IL-6 levels observed in the treatment group.

Vinblastine and vincristine, though primarily recognized as chemotherapeutic agents, also modulate inflammation by interfering with microtubule formation and inhibiting immune cell proliferation. Their presence in *C. roseus* may synergize with antioxidant compounds to suppress inflammatory

cascades.[22] These bioactive compounds make *C. roseus* a promising multi-target therapy against toxin-induced organ injury.

Caspase-3 is a critical executioner of apoptosis, activated by both intrinsic (mitochondrial) and extrinsic pathways. Once triggered, it cleaves cellular substrates, leading to DNA fragmentation and programmed cell death.[23,24] The elevated Caspase-3 levels in the cadmium-only group align with prior reports of cadmium-induced apoptosis in kidney tissues. In contrast, periwinkle extract treatment significantly lowered Caspase-3 levels, suggesting inhibition of apoptotic signaling pathways. This may be achieved by reducing oxidative damage and stabilizing mitochondrial membranes.[25,26]

Interestingly, vitamin E used as the positive control showed a slightly stronger effect on IL-6 suppression compared to periwinkle extract, although both treatments were equally effective in lowering Caspase-3 levels. Vitamin E is known for its lipid-soluble antioxidant capacity and its ability to modulate both NF-κB and Nrf2 pathways.[27,28] In previous studies, vitamin E has been shown to protect against various forms of nephrotoxicity, including those caused by heavy metals like cadmium and lead.[29,30]

Vitamin E, especially its tocotrienol form, is known to enhance antioxidant responses by stabilizing membrane lipids and activating Nrf2 transcription, thus boosting endogenous defense systems. Tocopherol also directly reduces NF-κB signaling, preventing further cytokine release.[31] This mechanism overlaps with those of flavonoids in *C. roseus*, suggesting a potential for combined or synergistic use in therapy. [32]

Other natural compounds such as nobiletin and celery extract have also demonstrated protective effects in nephrotoxic models, primarily through their antioxidant and anti-apoptotic actions. However, *C. roseus* offers a broader phytochemical profile, combining anti-inflammatory, anti-oxidative, and possibly cytoprotective properties in one plant. This diversity of action makes it an attractive candidate for integrative or complementary therapy.[33,34]

From a mechanistic standpoint, the suppression of IL-6 and Caspase-3 by *C. roseus* implies that the extract not only reduces upstream oxidative stress but may also interfere with downstream signaling involved in apoptosis. The activation of Nrf2 and inhibition of NF-κB are central to this protective mechanism. Moreover, periwinkle's multi-compound composition may allow it to act on multiple cellular targets simultaneously, o ffering broad-spectrum protection.

Despite these promising findings, this study has several limitations. The small sample size (n=5 per group) may limit statistical power and generalizability. Additionally, only two biomarkers IL-6 and Caspase-3 were measured. Including other inflammatory (e.g., TNF-α) or oxidative stress markers (e.g., MDA, GSH) would provide a more comprehensive view of the extract's protective effects. Future studies should investigate different dosages, time frames, and histopathological correlates, and potentially isolate specific active compounds from the extract.

In conclusion, *Catharanthus roseus* extract significantly reduced IL-6 and Caspase-3 levels in cadmium-exposed mice, indicating strong anti-inflammatory and anti-apoptotic activity. These effects, while slightly less pronounced than vitamin E in reducing inflammation, were comparable in mitigating apoptosis. With its diverse bioactive components and multi-pathway modulation, *C. roseus* holds promise as a natural nephroprotective agent against heavy metal toxicity. Further studies are warranted to explore its mechanisms in greater depth and its potential for clinical applications.

CONCLUSION

This study demonstrates that *Catharanthus roseus* extract effectively reduces Interleukin-6 and Caspase-3 levels in the kidneys of cadmium-exposed

mice, indicating strong anti-inflammatory and anti-apoptotic properties. While its effect on inflammation was slightly less pronounced than that of vitamin E, both treatments showed comparable efficacy in minimizing apoptosis. These findings highlight the potential of *C. roseus* as a natural nephroprotective agent. Beyond experimental evidence, the results suggest practical applications in the development of herbal-based therapies for heavy metal-induced kidney damage. Its multi-target bioactive components could serve as a complementary or alternative treatment strategy, especially in settings where synthetic antioxidants are limited.

Future research should explore dose-response relationships, long-term safety, and the specific roles of individual phytochemicals such as vincristine, vinblastine, and flavonoids. Additional studies integrating histopathological analysis and broader biomarker panels will help clarify the full therapeutic potential and mechanisms of action of this plant.

AUTHORS' CONTRIBUTIONS

Qisthy Kurrota Aini; Investigation, Data curation, Writing-Original draf, Conceptualization; Agung Putra: Reviewing, Supervision; Chodidjah: Supervision, Validation, Reviewing

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DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals.

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