

Effect Of Celery (*Apium graveolens L.*) Extract Administration On Kidney Function Of White Rats (*Rattus Norvegicus*) Wistar Strains Exposed To Lead

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Abstract.

Lead significantly affects health because of its sensitivity to the central nervous system and rapid absorption rate. The phytochemicals included in celery, such as flavonoids, may mitigate lead-induced kidney damage. This research aimed to determine how administering celery extract affected the expression of the Caspase-3 gene and the kidney TNF- α gene in *Rattus Norvegicus* Wistar white rats exposed to lead. The research method true experiment examined the effects of 80 mg/day celery extract on Caspase-3 gene expression and renal TNF- α gene expression in rats exposed to lead. Each of the three groups will include six rats; there will be eighteen male Wistar rats in the study. The study used SPSS 26.0 and the Saphiro Wills test to analyze data on TNF- α and Caspase 3 expression in rats exposed to lead acetate (Pb). The results showed a normal distribution for the negative control group (K-) and the positive control group (K+) and a normal distribution for the treatment group (P). However, the paired t-test showed significant differences in all treatment groups ($p < 0.05$), indicating a normal distribution in the data. The study concludes the impact of celery extract on kidney function in lead-exposed Wistar rats, revealing various active compounds like flavonoids. Despite insignificant caspase 3 reduction, flavonoids showed potential in mitigating lead-induced kidney damage.

Keywords: Lead, celery extract, kidney and Caspase-3, and TNF- α .

I. INTRODUCTION

Lead is a poisonous, indecomposable substance that accumulates rapidly in living organisms and can cause serious illnesses[1]. It dissolves quickly and is absorbed through respiration, food, and drink, affecting almost every organ. Children are particularly susceptible to lead exposure, which can lead to neurological disorders like headaches, nausea, tremors, gum buildup, and anemia due to interference with heme biosynthesis [2], [3], [4], [5]. Every year, lead poisoning affects an estimated 0.6 million children suffering from mild to severe mental impairment, according to the World Health Organisation [6]. Developing nations are home to 99% of the 600,000 new cases of mental retardation in children each year, which is directly attributable to lead exposure in childhood [7], [8]. Lead exposure causes 9.3 million DALYs, 0.5% premature deaths, 2.5% heart attacks, OS, and 12.4% intellectual impairment in developing nations, despite the phase-out of lead-containing fuels. An estimated 85 percent of the world's lead demand is met by the lead manufacturing and ULAB sectors, which drive up lead output worldwide [9], [10]. The production and recycling of lead-acid batteries significantly contribute to lead pollution[11]. Lead, a heavy metal found naturally in Earth's crust, is often combined with other elements to form compounds. Industrial use of lead-containing exhaust emissions poses health threats[12]. Primary lead comes from mined ore, recycled scrap metal, or batteries, while secondary lead comes from lead-acid batteries[13], [14]. The building industry uses lead batteries, alloys, soldering, and acid-resistant materials. Lead-induced anemia results from heme biosynthesis inhibition and shortens erythrocyte life span. [15].

Lead is absorbed by body parts and enters the blood, with blood lead concentration being the most reliable exposure indicator. Over 95% of blood lead is bound to erythrocytes, causing damage to organs like the liver, kidneys, heart, and male gonads and affecting the immune system [16]. Lead poisoning disrupts enzyme and essential cation function, leading to multi-systemic symptoms. It damages renal tubules, causing aminoaciduria, hypophosphatemia, and glycosuria. Lead also affects liver function, detoxifies xenobiotics,

alters tryptophan metabolism, and increases serotonin and indole acid hydroxy acetic acid in the brain, impairing neurotransmitter function [17]. The study [18] shows that lead (Pb) accumulation in female rats increases with age, with Pb levels in the bones of animals in different groups increasing in order. However, offspring born to mothers who consumed contaminated food showed higher lead concentrations in their bone tissue. This suggests that contamination of maternal milk is likely a contributing factor to the accumulation of Pb levels in offspring bones, as milk collected from the mother group revealed Pb presence (0.03 g g⁻¹ milk). A study found that control rats' liver parenchyma had a standard structure with cords and hepatocytes. However, treated rats showed significant degenerative changes, including diffuse hepatic disorganization, vascular congestion, and dilatation of sinusoid capillaries, central veins, and portal spaces. Infiltration and nuclear chromatin fragmentation were observed [19]. Organs, including the bones, liver, and kidneys, can be harmed over time by lead exposure. Nevertheless, there needs to be more studies in Indonesia about celery's (*Apium graveolens L.*) potential to lower blood lead levels or, more particularly, in the kidneys, which is the focus of this investigation. The researchers were inspired to do a lab experiment using lead-exposed white rats (*Rattus Norvegicus*) Wistar strain to determine the impact of celery extract on the expression of the Caspase-3 gene and the kidney TNF- α gene.

II. METHODS

This study describes laboratory experimental research, also known as a true experiment [20]. It examined the effects of administering celery (*Apium graveolens L.*) extract on the expression of the Caspase-3 gene and the renal TNF- α gene in rats exposed to lead. The rats used were white rats of the *Rattus Norvegicus* Wistar strain. Researchers considered the 3R Principle—Replacement, Reduction, and Refinement—when deciding the sample size for male Wistar strain white rats (*Rattus Norvegicus*) [21]. Each of the three groups will include six rats; there will be eighteen male Wistar rats in the study. Here is the formula that is used to compute the sample size according to Federer:

$(t - 1)(n - 1) \geq 15$	Description:
$(4 - 1)(n - 1) \geq 15$	t: Number of test groups
$3n - 3 \geq 15$	n: Sample size per group
$3n \geq 18$	
$n \geq 6$	

A variable is any observable quality that differs among the individuals or groups under study [22]. This research assessed factors such as celery extract, TNF- α , and Caspase-3. An independent variable is directly or indirectly related to a dependent variable whenever a dependent variable is studied. Anything that may be seen to alter throughout the study topics is considered a variable [23]. When determining the dosage earlier in the investigation, the dosage of celery extract was established by reviewing relevant literature. Past studies have shown that protecting kidney function can be achieved by taking 300 mg/kgBB of celery extract orally once daily. So, 400 mg/kgBB/day was used in this research. Oral administration of 80 mg/day of celery extract was administered to the rats in this study, which had body weights ranging from 180-250 gr. The study begins with test animals acclimating to a new location, climate, condition, or atmosphere.

For celery extract ointment, 750 grams of celery leaves were dried, diced, extracted, filtered, macerated, evaporated, and refrigerated at 2-8°C. A 95% ethanol extract was macerated. To determine dose A literature investigation determined the celery extract dose before research. According to studies, oral Celery extract at 300 mg/kgBB/day preserves renal function. Therefore, this study employed 400 mg/kgBB/day. This study used 80 mg/mouse/day celery extract for rats weighing 180-250 gr. The individuals' lead exposure and therapy altered the aspirin dose one month after its introduction, but it did not affect its effectiveness for 14 days. Adjusted aspirin doses were 200 mg/kg BW, 100 IU vitamin E, and 400 mg/kg BW. 3.6.5 Quantitative tnf- α and caspase-3 expression investigation using rt-PCR. Data analysis from each group was then analyzed with the help of SPSS (Statistic of Package for Social Science) 26.0. for windows [24]. Because the number of samples is less than 50, the statistical test used is the normality test using Shapiro Wilk and continued with the paired T-test [25].

III. RESEARCH RESULTS AND DISCUSSION

RESEARCH RESULT

The study investigated the impact of celery (*Apium graveolens L.*) extract on Caspase-3 gene expression and renal TNF- α gene expression in male Wistar strain white rats exposed to lead. The extract was extracted from celery plants, blended into dry powder, macerated with 95% ethanol, filtered, and macerated again. The resulting extract was then evaporated to form a thick extract, which was stored in a refrigerator at 2-8°C until treatment was administered. The study provides valuable insights into the potential of celery extract in various health applications. Celery leaf extract, rich in phenolic compounds, minerals, vitamins, and antioxidant properties, is widely used due to its easy cultivation and numerous properties.

Table 1. Celery Extract Phytochemical Screening Results

Secondary Metabolites	Result
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Steroids/Triterpenoids	+
Glycosides	-

Phytochemical tests have confirmed the presence of active compounds like alkaloids, flavonoids, saponins, tannins, and steroids in the extract. These antioxidants benefit the body, making them valuable to any diet.

Table 2. Mean TNF- α Expression Levels In Mice Exposed To Lead Acetate (Pb)

Variables	Group			P value
	Control (-) n=5 Mean \pm SD	Control (+) n=5 Mean \pm SD	P n=5 Mean \pm SD	
TNF- α expression	0,536 \pm 0,161	4,047 \pm 0,015	2,658 \pm 0,130	
Saphiro Wilk	0,258	0,671	0,260	
Paired sample test				0,158

TNF- α is a cytokine produced by macrophages, which will significantly affect inflammation. The value of TNF- α expression in the negative control group (K-) will be used as a standard to determine whether an increase or decrease occurs due to the treatment effect. In the ileum of positive control rats (K+) induced with vitamin E orally at a dose of 100 IU can be seen to increase the expression of TNF- α with a value of 4,047. Then, in the treatment group (P), using celery extract orally at a dose of 400 mg/kg BW showed a decrease in TNF- α expression with a value of 2.658. The reduction in TNF- α expression proves that using celery extract containing flavonoids can reduce the effects of free radicals due to the induction of lead acetate in rats. The data generated in this study were analyzed using the Saphiro Wills test to determine normality and then analyzed using the paired t-test ($p < 0.05$) to see the significance in all groups.

Table 3. Normality Test Results Of TNF- α Expression

Group	Shapiro-Wilk		
	Statistic	df	Sig.
TNF- α	K-	.877	6 .258
	K+	.941	6 .671
	P	.878	6 .260

Based on this statistical analysis, it was found that the results of TNF- α expression in rats exposed to lead acetate (Pb) on day 14 were known to have normal distribution data with the results of the Shapiro Wilk test for the negative control group (K-) of 0.258, the positive control group (K+) of 0.671 and the treatment group (P) of 0.260 so ($p > 0.05$) that the data had a normal distribution. The paired t-test result of 0.158, where the value ($p > 0.05$) shows no significant difference in all treatment groups. The study involved mice with body weights ranging from 180-250 gr, given a dose of celery extract orally at 80 mg/mouse/day. Rats were adapted for one week, with healthy rats not exposed to lead acetate exposure and standard feed for 14 days. Negative control rats were induced with lead acetate orally at 200 mg/kg BW dissolved in 2mL

distilled water for 14 days, while positive control rats were induced with lead acetate orally at 200 mg/kg BW and vitamin E at 100 IU and treatment control rats were given celery extract orally at 400 mg/kg BW.

Table 4. Results of Celery Extract Administration on Caspase-3 Gene Expression and Data Analysis

Variables	Group			P value
	Control (-) n=5	Control (+) n=5	P n=5	
	Mean±SD	Mean±SD	Mean±SD	
Caspase 3	12,260 ± 0,120	9,418 ± 0,317	8,354 ± 0,022	
Saphiro Wilk	0,947	0,347	0,351	
Paired sample test				0,000

From the table above, the negative control group (K-) with the treatment of rats induced by lead acetate orally with a dose of 200 mg/kg BW and still given standard feed got the highest mean caspase three and standard deviation of $12,260 \pm 0,120$ compared to the positive control group (K+) rats induced by lead acetate orally with a dose of 200 mg/kg BW and simultaneously given vitamin E orally with a dose of 100 IU and still given standard feed the day showed a decrease in the mean caspase three and standard deviation of $9,418 \pm 0.317$ and in the Treatment Group (P) rats induced lead acetate orally with a dose of 200 mg/kg BW and simultaneously given celery (*Apium graveolens L.*) extract orally with a dose of 400 mg/kg BW and still given standard feed showed a better decline than other groups with an average value and standard deviation of 8.354 ± 0.022 . Although the level of caspase three reduction is not so significant, in the negative control treatment (K-), positive control (K+), and the treatment group (P), there are differences in the average results obtained. The data generated in this study were analyzed using the Saphiro Wills test to determine normality and then analyzed using the paired t-test ($p < 0.05$) to see the significance in all groups.

Table 3. Normality Test Results of Caspase 3

Group	Shapiro-Wilk		
	Statistic	df	Sig.
Caspase3	K-	.979	6 .947
	P	.896	6 .351
	K+	.895	6 .347

Based on this statistical analysis, it was found that the results of Caspase 3 expression in rats exposed to lead acetate (Pb) on day 14 were known to have normal distribution data with the results of the Shapiro Wilk test for the negative control group (K-) of 0.947, the positive control group (K+) of 0.347 and the treatment group (P) of 0.351 so ($p > 0.05$) that the data had a normal distribution. The paired t-test results are 0.00, where the value ($p < 0.05$) shows significant differences in all treatment groups.

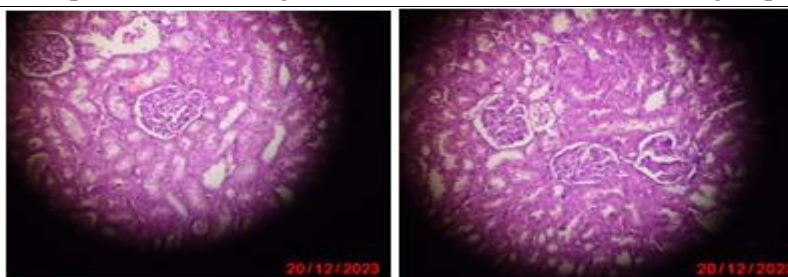


Fig 1. Kidney Histopathology of Negative Control Group

There is severe necrosis (blue arrow) as well as severe inflammation (green arrow)

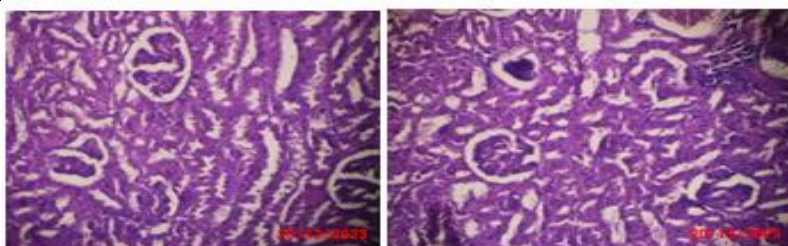


Fig 2. Kidney Histopathology of Positive Control Group
There is moderate necrosis (yellow arrow) and bleeding.

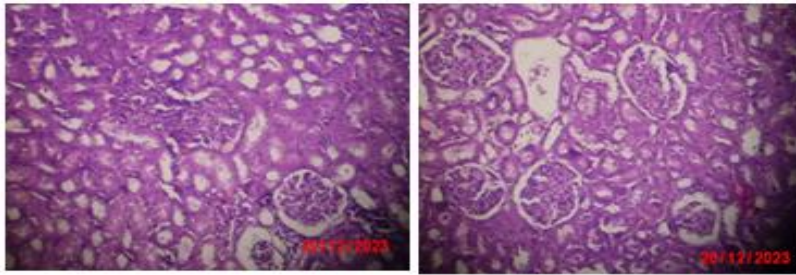


Fig 3. Kidney Histopathology of Treatment Group
There is moderate necrosis (yellow arrow)

The above picture shows bleeding; the cause of kidney damage is due to the toxic effects of Pb acetate, which causes oxidative stress and damage to cell membranes, DNA, and antioxidants[26]. ROS (reactive oxygen species) outnumber antioxidants, leading to cell death and tissue damage[27]. The lipophilic nature of lead causes it to react with the lipid bilayer, triggering cell damage quickly. In the treatment group (P), kidney function is moderately affected, possibly due to the administration of celery extract, which can help recover kidney function damaged by lead acetate exposure.

RESEARCH DISCUSSION

The study investigates the impact of celery (*Apium graveolens L.*) extract on Caspase-3 gene expression and renal TNF- α gene expression in white rats exposed to lead, highlighting the potential oxidative stress caused by lead. Phytochemical tests on celery leaf extract revealed its antioxidant benefits, including alkaloids, flavonoids, saponins, tannins, and steroids. These compounds are beneficial for the body [28]. The extract also showed positive effects on kidney function in rats exposed to lead acetate, indicating its potential for further research. Caspases are vital proteases that activate and mediate apoptotic cell death through protein cleavage cascades. Apoptotic caspases are hierarchically organized into apical caspases (caspase-2, -8, -9, and -10) and effector caspases (caspase-3, -7, and -6) [29]. An extrinsic stimulus can induce apoptosis via death receptors (such as tumor necrosis factor/TNF or FAS receptors) that specifically activate caspase-8 or an intrinsic stimulus (such as expression of the BCL2 family BH3-only protein BIM or puma) that leads to mitochondrial depolarization and activation of caspase-9 [30].

In the test to see the results of the Caspase -3 gene, the negative control group (K-) with the treatment of rats induced lead acetate orally with a dose of 200 mg/kg BW and still given standard feed got the highest mean caspase three and standard deviation of $12,260 \pm 0,120$ compared to the positive control group (K+) rats induced lead acetate orally with a dose of 200 mg/kg BW and simultaneously given vitamin E orally with a dose of 100 IU and still given standard feed the day showed a decrease in the mean caspase three and standard deviation of $9,418 \pm 0.317$ and in the Treatment Group (P) rats induced lead acetate orally with a dose of 200 mg/kg BW and simultaneously given celery extract orally with a dose of 400 mg/kg BW and still given standard feed showed a better decline than other. There are variances in the average findings obtained from the negative control treatment (K-), positive control (K+), and the treatment group (P), even if the amount of caspase three decrease is not very considerable. One of the cytokines, TNF- α , controls inflammation, homeostasis, and the immune system. The immune system can ward off infections, construct lymphoid organs, decrease inflammation, repair damaged tissues, and forestall tumor development. Its harmful effects include swelling, activation of the vascular endothelium, multiplication of immune cells, and harm to tissues.

To establish whether the treatment impact causes an increase or reduction, the value of TNF- α expression in the negative control group (K-) will be utilized as a benchmark. The ileum of rats given 100 IU of vitamin E orally showed an increase in TNF- α expression with a value of 4.047. Next, a reduction in TNF- α expression of 2.658 was seen in the treatment group (P) when 400 mg/kg BW of celery extract was taken orally. Reducing the effects of free radicals caused by the induction of lead acetate in rats can be achieved by

employing celery (*Apium graveolens L.*) extract containing flavonoids, as evidenced by the decrease in TNF- α expression. The hepatology picture revealed bleeding, which was caused by exposure to harmful compounds, specifically Pb acetate. In this study, the renal organs developed necrosis due to oxidative stress and the detrimental effects of Pb acetate. When reactive oxygen species (ROS) outnumber antioxidants, a condition known as oxidative stress develops in cells, treatment group P, on the other hand, has moderate necrosis in terms of renal function. This is likely because celery extract, when administered, can effectively restore kidney function that has been compromised by lead acetate exposure. In this research, rats exposed to lead acetate did not show a significant decrease in caspase three and TNF- α levels after being given celery extract. However, histological observations revealed that the group given celery extract had moderate necrosis in their kidney function compared to the group that did not receive celery extract. This agrees with the findings of a study [31], which found that while celery leaf extract did not mitigate the creatinine-induced kidney damage in male white rats, it did so in histopathology, with the histopathology results improving with increasing dosage.

IV. CONCLUSION

The kidney function of Wistar strain white rats exposed to lead was studied intriguingly using celery (*Apium graveolens L.*) extract. Various active chemicals, including steroids, alkaloids, flavonoids, saponins, and tannins, were identified in the phytochemical testing of celery leaf extract. These substances shield cells from free radical damage and offer substantial antioxidant advantages to the body. Even though caspase three levels did not drop statistically, it is intriguing to see how the treatment group, negative control, and positive control groups fared on average in the test. The ability of flavonoids to reduce the effects of lead acetate-induced free radicals in mice was demonstrated in this study by the fact that TNF- α expression decreased following the administration of celery extract. Based on the findings of this study, we now know more about how celery extract may mitigate the harmful effects of lead on kidney health.

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REFERENCES

- [1] M. Balali-Mood, K. Naseri, Z. Tahergorabi, M. R. Khazdair, and M. Sadeghi, "Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic," *Front Pharmacol*, vol. 12, no. April, pp. 1–19, 2021, doi: 10.3389/fphar.2021.643972.
- [2] D. Bellinger, "The Protean Toxicities of Lead: New Chapters in a Familiar Story," *Int J Environ Res Public Health*, vol. 8, no. 7, pp. 2593–2628, Jun. 2011, doi: 10.3390/ijerph8072593.
- [3] N. Rees and R. Fuller, *The Toxic Truth Children's Exposure to Lead Pollution Undermines a Generation of Future Potential*, 2nd ed. New York: UNICEF and Pure Earth, 2020.
- [4] J. Schwartz, "Low-Level Lead Exposure and Children's IQ: A meta-analysis and Search for a Threshold," *Environ Res*, vol. 64, pp. 42–45, 1994.
- [5] S. Hou *et al.*, "A clinical study of the effects of lead poisoning on the intelligence and neurobehavioral abilities of children," *Theor Biol Med Model*, vol. 10, no. 1, pp. 1–9, 2013, doi: 10.1186/1742-4682-10-13.
- [6] P. J. Landrigan *et al.*, "The Lancet Commission on pollution and health," *The Lancet*, vol. 391, no. 10119, pp. 462–512, Feb. 2018, doi: 10.1016/S0140-6736(17)32345-0.
- [7] D. Bellinger, "The Protean Toxicities of Lead: New Chapters in a Familiar Story," *Int J Environ Res Public Health*, vol. 8, no. 7, pp. 2593–2628, Jun. 2011, doi: 10.3390/ijerph8072593.
- [8] A. E. Charkiewicz and J. R. Backstrand, "Lead toxicity and pollution in Poland," *Int J Environ Res Public Health*, vol. 17, no. 12, pp. 1–16, 2020, doi: 10.3390/ijerph17124385.
- [9] M. H. Forouzanfar *et al.*, "Global, regional, and national comparative risk assessment of 79 behavioral, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015," *Lancet*, vol. 388, no. 10053, p. 1659, Oct. 2016, doi: 10.1016/S0140-6736(16)31679-8.

- [10] K. L. Hipkins, B. L. Materna, M. J. Kosnett, J. W. Rogge, and J. E. Cone, "Medical surveillance of the lead exposed worker: Current Guidelines," *AAOHN Journal*, vol. 46, no. 7, pp. 330–339, 1998, doi: 10.1177/216507999804600705.
- [11] L. D. Grant, "Lead and Compounds," in *Environmental Toxicants: Human Exposures and Their Health Effects: Third Edition*, 4th ed., M. Lippmann and G. D. Leikauf, Eds., John Wiley & Sons, Inc, 2020, pp. 757–809. doi: 10.1002/9780470442890.ch20.
- [12] S. Kendall, S. Redshaw, S. Ward, S. Wayland, and E. Sullivan, "Systematic review of qualitative evaluations of reentry programs addressing problematic drug use and mental health disorders amongst people transitioning from prison to communities," *Health Justice*, vol. 6, no. 1, p. 4, Dec. 2018, doi: 10.1186/s40352-018-0063-8.
- [13] J. P. A. P. A. P. G. G. G. G. A. S. A. S. A. R. K. Mandakini Kshirsagar Mandakini Kshirsagarand Jyotsna Patil, "Biochemical effects of lead exposure and toxicity on battery manufacturing workers of Western Maharashtra (India): with respect to liver and kidney function tests.," vol. 8, no. 2, pp. 107–114, 2015.
- [14] A. Patil *et al.*, "Effect of Lead (Pb) Exposure on the Activity of Superoxide Dismutase and Catalase in Battery Manufacturing Workers (BMW) of Western Maharashtra (India) with Reference to Heme biosynthesis," *Int J Environ Res Public Health*, vol. 3, no. 4, pp. 329–337, Dec. 2006, doi: 10.3390/ijerph2006030041.
- [15] J. P. A. P. A. P. G. G. G. G. A. S. A. S. A. R. K. Mandakini Kshirsagar Mandakini Kshirsagarand Jyotsna Patil, "Biochemical effects of lead exposure and toxicity on battery manufacturing workers of Western Maharashtra (India): with respect to liver and kidney function tests.," vol. 8, no. 2, pp. 107–114, 2015.
- [16] U. S. D. of H. and H. Services, "Agency for Toxic Substances and Disease Registry - ATSDR.," 1999, Accessed: Mar. 23, 2024. [Online]. Available: <http://www.atsdr.cdc.gov/atsdrhome.html>
- [17] A. Patil *et al.*, "Effect of Lead (Pb) Exposure on the Activity of Superoxide Dismutase and Catalase in Battery Manufacturing Workers (BMW) of Western Maharashtra (India) with Reference to Heme biosynthesis," *Int J Environ Res Public Health*, vol. 3, no. 4, pp. 329–337, Dec. 2006, doi: 10.3390/ijerph2006030041.
- [18] C. R. B. Nascimento, W. E. Risso, and C. B. dos R. Martinez, "Lead accumulation and metallothionein content in female rats of different ages and generations after daily intake of Pb-contaminated food," *Environ Toxicol Pharmacol*, vol. 48, pp. 272–277, Dec. 2016, doi: 10.1016/j.etap.2016.11.001.
- [19] S. A. Zidi I, "Hepatotoxic Effects of Lead Acetate in Rats: Histopathological and Cytotoxic Studies," *J Cytol Histol*, vol. 05, no. 05, 2014, doi: 10.4172/2157-7099.1000256.
- [20] S. Notoatmodjo, *Metodologi Penelitian Kesehatan*, 3rd ed. Jakarta: Rineka Cipta, 2022.
- [21] L. V. Kendall *et al.*, "Replacement, Refinement, and Reduction in Animal Studies With Biohazardous Agents," *ILAR J*, vol. 59, no. 2, pp. 177–194, 2018, doi: 10.1093/ilar/ily021.
- [22] J. W. Creswell and J. D. Creswell, *Research design: Qualitative, quantitative, and mixed methods approaches*. Sage publications, 2017.
- [23] B. Suwarno and A. Nugroho, *Kumpulan Variabel-Variabel Penelitian Manajemen Pemasaran (Definisi & Artikel Publikasi)*, 1st ed. Bogor: Halaman Moeka Publishing, 2023.
- [24] S. Santoso, *Panduan Lengkap SPSS 26*. Elex Media Komputindo, 2020.
- [25] P. Mishra, U. Singh, C. M. Pandey, P. Mishra, and G. Pandey, "Application of Student's t-test, Analysis of Variance, and Covariance," *Ann Card Anaesth*, vol. 22, no. 4, p. 407, 2019, doi: 10.4103/ACA.ACA_94_19.
- [26] M. H. Little, *Kidney development, disease, repair and regeneration*. Academic Press, 2015.
- [27] Z. He *et al.*, "Reactive oxygen species (ROS): utilizing injectable antioxidative hydrogels and ROS-producing therapies to manage the double-edged sword," *J Mater Chem B*, vol. 9, no. 32, pp. 6326–6346, Aug. 2021, doi: 10.1039/D1TB00728A.
- [28] G. A. Engwa, "Free radicals and the role of plant phytochemicals as antioxidants against oxidative stress-related diseases," *Phytochemicals: source of antioxidants and role in disease prevention. BoD—Books on Demand*, vol. 7, pp. 49–74, 2018.
- [29] E. A. Slee *et al.*, "Ordering the Cytochrome c-initiated Caspase Cascade: Hierarchical Activation of Caspases-2, -3, -6, -7, -8, and -10 in a Caspase-9-dependent Manner," *J Cell Biol*, vol. 144, no. 2, pp. 281–292, 1999, doi: <https://doi.org/10.1083/jcb.144.2.281>.
- [30] S. McComb *et al.*, "Efficient apoptosis requires feedback amplification of upstream apoptotic signals by effector caspase-3 or -7," *Sci Adv*, vol. 5, no. 7, Jul. 2019, doi: 10.1126/sciadv.aau9433.
- [31] N. Novelli, "Pengaruh Pemberian Ekstrak Daun Seledri (*Apium graveolens L.*) Selama 7 Hari Terhadap Fungsi Ginjal Tikus Putih Jantan Yang Diinduksi Gentamisin," Oct. 2019.